Studies on fungal diseases of Arhar (*Cajanus cazan*)

Thesis

Submitted for the Degree of Doctor of Philosophy in Science

by

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DEDICATED

TO

MY

BELOVED

PARENTS

WHO DEVOTED

THEIR ENTIRE LIFE

FOR MY SUCCESS

CERTIFICATE

Certified that the thesis embodies results of original research work and study carried out under my supervision by Mr. Ashok Kumar Singh M.Sc. (Ag.) Plant Pathology.

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PREFACE

The thesis embodies research work on **"Studies on fungal diseases of Arhar (*Cajanus cajan*)"**. The work carried out by me from September 2000 to September 2002 in the Bhargva Agricultural Laboratory, Garden and Farm of Botany Department, University of Allahabad and a comprehensive survey of Allahabad and its adjacent regions.

The thesis embodies researches with studies on some seed-mycoflora isolated from Arhar seeds and Fungi isolated from soil samples fungi identified and morphological characters observed. The thesis contains work on Isolation and Pathogenicity Tests, Pysiological studies, studies of some isolated mycoflora with their respective host seeds and control studies.

The thesis is organised into Eight Chapters. First chapter deals with Introduction followed by Materials and Methods (Chapter - 2) Isolation and Pathogenicity Tests are discussed in Chapter - 3. Physiological Studies and Control Studies are given in Chapter - 4 and Chapter - 5 respectively.

A detailed Discussion on the basis of the present investigation and the Summary are given in Chapter - 6 and Chapter - 7. The bibliography containing the references cited in the text is appended in the end (Chapter-8). Abstract of the present work is also submitted along with the thesis separately.

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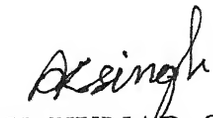

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"People need food, not production statistics, and crop is not food until it is eaten. A programme to reduce storage losses, probably could result in a 10 – 20 percent increase in available food now in some of the developing countries, and might also assure that whatever increases occur in production in the future could be used for the nourishment of people, not for feeding pests"

— Christensen and Kaufman

Chapter 1

Introduction

Healthy Crops of Arhar (*Cajanus cajan*)

INTRODUCTION

"A disease is borne in the seed, in the sense that potentially, it is brought forth or given support by the seed".

— Neergaard (1977)

Pulses provide essential ingredients to our daily diet and improve the nutritional quality of the predominantly cereal-based diets of large segments of Indian population, particularly the vegetarians. They contain about two to three times more protein, four times the riboflavin and ten times the thiamine than that of cereals. Pulses are also relatively rich in two essential micro-nutrients, viz. calcium and iron than cereals. In a country like India where a large population is vegetarian, the cheap and best source of protein are still pulses. On an average, pulses contain 20-25% protein on dry seed basis, which is almost 2.5-3.0 times of the value normally found in the cereals. The pigeonpea contains 22.9 percent protein. (The Hindu Survey 2002).

On account of a balanced amino acid composition of cereals and pulses protein blend, which matches the milk protein, the importance of pulses in vegetarian diet can not be over emphasized.

Since pulses are cheaper than meat (animal protein) they are often referred to as 'poor man's meat' in developing countries like India. The concerted efforts should be made to enhance the production and productivity of pulses, which will in turn ensure more availability of per capita of pulses.

Thus, it will ensure nutritional security to the poor masses of the country. Besides being a rich source of protein, they are also important for sustainable agriculture as they improve physical, chemical and biological property of soil and function as "mini nitrogen factory".

Pulses occupy 68.32 million hectare area and contribute 57.51 million tonnes to the world's food basket. India shares 35.2% area and 27.65% of the global production. Percent contribution of pulses in total food grain production in India has declined during the last three decades.

The Indian Council of Medical Research has recommended pulses intake of 50 g/capita/day. The cost of protein for pulses comes around Rs. 100/kg whereas for eggs, milk and fish/meat the cost is around Rs. 200, Rs. 300, Rs. 450/kg, respectively.

On the basis of food characteristic demand system, the demand projections for pulses for the years 2005, 2010 and 2015 are 20.0 million tonnes, 23.3 million tonnes and 27.0 million tonnes respectively. (Chaturvedi & Ali 2002).

The production of pulses is insufficient, today to meet the present day requirements. According to an estimate of World Health Organisation, the minimum recommended requirement of pulses is 80 g for an individual and we are able to take only 40 g. The present production has to be increased from 12.0 million tonnes to 23.8 million tonnes by the end of twentieth century to cater the need of growing population (Lal, 1986). It can be achieved by minimizing the incidence of losses during threshing, transport, processing and storage. The expert committee, Government of India in their interim report reported a total loss of 9.5 percent, of which 80 percent was due to insects, rodents and microorganisms in storage and remaining during transit and processing.

Among pulses, pigeon pea (*Cajanus cajan* (L.) millsp.) which is commonly known as arhar, tur, redgram, cangopea and gandul, holds an important position in Indian subcontinent. Besides India, it is grown in south-east Asia, Africa and America. It is woody, short-lived perennial shrub of sub family Papilionaceae of Leguminaceae family. According to statistics compiled by Food and Agriculture Organisation, this crop covers 29 lakh hactares of world's acreage with an average yield of 684 kg/hectare during 1975-80. It is grown as backyard crop in most of the American countries, whereas, in Dominican Republic and Puerto Rico, it is grown commercially (Kannaiyan *et al.*,1984).

In India, it is cultivated on total acreage of 35.259 lakh hectares with the production of 26.661 lakh tonnes and average yield of 756kg per hectare. This

crop is grown almost in every state of India, chiefly being Maharashtra with an acreage of 8.969 lakh hectares; Karnataka, 4.952 lakh hectares; Madhya Pradesh 4.797 lakh hectares; Uttar Pradesh 4.729 lakh hectares; Gujrat 3.369 lakh hectares; Andhra Pradesh 3.357 lakh hectares; Orrisa, 1.563 lakh hectares. The state of Uttar Pradesh has an unique distinction of contributing about one forth of the total production in our country (Anonymous,1990). Pigeon pea is cultivated in black or loamy soils intercropped with sorghum, pearlmillet, maize, groundnut, urd, mung and lobia.

Pigeon pea is extensively used as dal, its green seeds and tender pods are used as vegetable. The green leaves and top of plants are fed to animals or utilized as green manure while dry stalks obtained after threshing are used for fuel, making baskets or as thatching material

Singh and Prasad (1977) noticed decrease in starch content of pigeonpea seeds by *Aspergillus flavus* after 15 days of incubation. Out of three soluble sugars, like glucose, fructose and sucrose, the former and the later were completely utilized by this fungus. The concentration of citric, tartaric and oxalic acids was increased in the infested seeds as compared to that in the healthy ones. Amino acids viz., glutamic acid, serin/glycine, leucine/isoleucins, lysine, triptophan and tyrosine get reduced considerably ofter 15 days of incubation.

Singh, (1988) observed, a sever leaf-spot and blight symptoms on a local variety of pigeonpea; caused by *Fusarium semitectum.* Reddy, *et. al.*, (1989), Visits to pigeonpea plots which were affected stem canker caused by *Macrophomina phaseolina* Ashby, reported that is a very common and serious disease. The symptoms for the disease ranged from restricted spindle shaped lesions on the stems of the plants, without any apparent damage; to extend lesions causing drying of the hole plant.

Nene, (1985) reported fifteen fungal diseases in pigeonpea crops. Saksena and Kumar, (1971) observed incidence of *Phyllosticta cajani*, which appears in July and persists throughout the crop season, infection varies from 4.3 to 31.8%. Infection is high (21.3-31.8%) in July to October, (Temp. is 26-30°C) and Av. R.H. 73-89%). Mehta and Sinha, (1982) reported a new leaf-spot disease of Arhar (*Cajanus cajan*, L.) caused by *Alternaria alternata* from India.

This crop suffers from more than fifty diseases caused by fungi, bacteria, viruses, nematodes and mycoplasma-like organisms (Chandra *et al.*, 1985) but only few of them viz. wilt, sterility mosaic, Phytophthora and Alternaria blight are major and destructive ones. According to an estimate, only wilt and sterility mosaic are known to cause losses to a tune of 113 million dollars annually in India during 1975-80 (Kannaiyan *et al.*, 1984). Other economically important diseases are Phyllosticta leaf spot, Rhizoctonia blight and yellow mosaic. Among them, Phyllosticta leaf spot often assumes in destructive nature during rainy season and

causes heavy defoliation which reflects ultimately loss in yield (Saksena and Kumar, 1971). Alternaria blight, particularly in rabi sown crop also assumes severe proportions off and then in North-Eastern parts of India (Mehta and Sinha, 1982).

Many fungi are serious parasites and they reduce production both quantitatively and qualitatively. Other fungi including saprophytes and very weak parasites may lower the quality of seeds by discolouration. Pulse disease are caused by two types of fungi one is seed borne and other is soil borne fungi. The major abstracts in the way of increase pulse production are the diseases responsible for the serious yield reduction and contribute substantially to instability of production.

Pathogen responsible for reduction in germination percentage of seeds and an increase in the pre and post emergence rots of various pulse crops. Some fungi secrete mycotoxins which are responsible for the reduction in seed germination and cause wilting of the seedlings and also effect seed viability (Suryanarayan, Bhombe and Ram Nath, 1963). These not only effect seed viability but are also harmful and dangerous for animal and human being, if consumed.

In addition, the fungal flora associated with seeds cause much damage to pigeonpea crop by producing seed rot, seedling mortality, leaf spots and blights, stem and/or petiole blight, stem canker and root rot. A variety of fungi invade

seeds before harvest and produce discolouration, malformation and spots on pods and seeds and other visible symptoms on plants. The seeds from such plants adversely affect seed germination and seedling vigour. Post-harvest spoilage of stored grains due to seed-borne fungi also cause biochemical changes in seed contents making them unfit for consumption and sowing purposes. Pre-mature harvesting and then its unscientific storage further promote the aggressiveness of the mycoflora by producing seed rot, seedling mortality and alteration in the seed constituents.

Soil is supposed to be a medium as a complex environment where fungi including other micro-organisms almost complete for their existence. The soil profile is made up of three layers called, A, B and C horizons. Horizon A is the surface soil rich in organic matter content where plant roots, fungi and micro-organisms are most dense and this stratum is subjected to leaching and intense competetion between microbes (Garrett, 1963; Dwivedi, 1966; Alexender, 1977; Fitzpatrick, 1980).

Garrett (1956) classified such fungi into two categories (1) soil inhabiting fungi including root parasites and mycorrhizal fungi. The fungi inhabiting the soil have been further classified into three categories (Burges 1965) : (i) Those which complete most of their life in soil (ii) Those which primarily attack plant materials but can continue growing in the soil at least for a time and (iii) Those which come to the soil accidentally.

The study of soil fungi started an early as 1886 when Adametz in Germany isolated several species of fungi in the course of his biochemical studies on soils. This work was, however, not followed up till 1902 when Oudemans and Koning isolated and described forty-five species of soil fungi from Holland, the majority of which were new to science. After this a large number of contributions were made from different parts of the world including Hagem (1907), Londner (1908), Dale (1912 and 1914), Jensen (1912), Harvey (1925), Jensen (1931), Gray and McMaster (1933), Gray and Taylor (1935), Campbell (1938), Garrett (1938 a, b), Tresner *et al.* (1954), Warcup (1955) and Durrell and Shields (1960).

In our country study of soil fungi started in 1915, when Shaw recorded four isolations from Pusa soils. Thakur and Norris (1928) described about twenty-five species from Madras soil with special reference to their power of cellulose decomposition and amonification. Chaudhuri and Sachar (1934), Chaudhuri (1939) and Chaudhuri and Umar (1939) described a number of fungi from the soils. Galloway (1936) made two hundred isolations from different plots, representing about thirty genera and allotted them to their approximately systematic positions. Ghatak and Roy (1939) isolated twenty-three fungi, belonging to seven genera from the paddy fields of Indian soils (Subramanian, 1951, 1952a, 1952b; Zachariah, 1949; Venkat Ram, 1954) collected from different regions in Southern India. Agnihotrudu (1972) isolated *F. semitectum, F. oxysporum, F. solani* and *F. stilboides* from the soils of coffee plantations.

Warcup (1950, 1955, 1957) in his extensive studies on soil fungi described at large number of forms throw new techniques including the well known soil plate method for qualitative as well as quantitative estimation of fungal population. Subramanian (1951, 1952) made extensive studies in soil fungi particularly on species of *Fusarium* from black cotton soil of Madras and he recorded and published soil fungi from Indian soil in 1952.

Soil is a sink for pathogenic and non-pathogenic genera of fungi. The relative abundance of both categories in soil depends on their behaviour against each other and the physico-chemical factors (Issoc., 1956).

The term 'rhizosphere' for the first time was introduced in 1904 by a German scientist Hiltner. It is the region of soil which is subjected to the influence of plant roots or in other words, the soil region adjacent to the plant root system. The significance of rhizosphere in relation to root diseases came in light in 1930 when Garrett evinced that the activities of the rhizosphere mycoflora affected the 'Take-all' fungus. Later researches on the root surface and rhizosphere microflora have been extended by Starkey (1929 a, b, c); Timonin (1940); Katznelson, (1965) ground the root surface and rhizosphere together and termed the 'root region'. Since then a good deal of work has been reviewed by Garrett (1956), Rovira (1965), Katznelson (1965), Rovira and Davey (1974) Brown (1974), Bowen and Rovira (1976), Dwivedi (1979), Roy *et al.* (1980) and Satyaprasad (1982). Rhizosphere was distinguished into (a) "Outer rhizosphere" (soil adhering to the

root) and (b) "inner rhizosphere" (root surface) by Poschenriedder (1930) and Graf (1930). The term 'rhizoplane' was introduced by Clark (1949). Rhizoplane is the region of external plant root surface and closely adhering particles of soil and plant debris.

Among the various physico-chemical properties of the soil, soil temperature, moisture content, aeration, soil texture, pore space, organic content, affect the activity of fungi and other micro-organisms (Warcup, 1951; Shaw, 1952; Dwivedi 1965; Alexander, 1977.

Based on the range of tolerance to temperature, the fungi can be grouped into (i) thermophilic fungi surviving at or above 40°C (ii) mesophilic surviving between 10°C and 40°C and (iii) psychrophilic-thriving at or below 10°C. Stanley and Nimmo (1979) reported that *Cercospora nicotiana* grew at 10-40°C. Hasija (1970) found the *Alternaria citri* grew at 15-35°C needed higher temperature (40-50°C).

Soil water is one of the factors influencing the growth and survival of soil play an important role (Griffin, 1963a, b, 1969; Rovira, 1965; Cook and Flentje, 1967; Cook and Papendick, 1970).

Distribution of soil mycoflora is affected by pH of soil (Griffin, 1972; Bissett and Parkinson, 1979 a, b, c). The species of a fungus gradually changes

with differences in vegetational cover and soil characteristics (Wohlrab *et al.*, 1963; Wicklow, 1973; Wicklow and Whittingham, 1978.

The dynamism of metabolic interations between the rhizosphere micoflora and green plant varies with type of species or plant (Nicholas, 1965). Moreover, the outer part of the root system resulting in the inhancement of rhizosphere population of microbes in soil.

The considerable variations in the fungal population of rhizosphere soil with the age and kind of plants have been reported by Peterson (1959). Rouatt and Katznelson (1961). Effect of rhizosphere region also depends either directly or indirectly on the moisture content and temperature of the environment (Timonin, 1940).

Wilt of pigeon pea, which is caused by *F. oxysporum, F. udum* is one of the most destructive diseases which is prevalent all over the country. It causes much damage in parts of Bihar, Maharashtra, Madhya Pradesh and Uttar Pradesh. So far practically only a little work has been done on *Fusaria* associated with plants of economic importance or on pulses. It was, therefore, decided to undertake some studies on their role in soils of garden plants, fruit trees and crops.

In a preliminary study, *Fusaria* of common occurrence in the soils of Allahabad were isolated and an ecological studies of twelve different fields namely (1) Chaka Block (2) Bara Region (3) Karchana Region (4) Koraon Region (5)

Meja Region (6) Handia Region (7) Phoolpur Region (8) Soraon Region (9) Begum Sarai (10) Khushroo Bagh (11) Chandra Shekhar Azad Park (12) University Farm, University of Allahabad and it's adjacent areas were carried out.

Finally a detailed study consisting of pathological and survival of four species of *Fusarium* viz., *F. acuminatum* Ellis and Everh., *F. oxysporum* Schlecht., *F. solani* (Mart.) Sacc. and *F. equiseti* isolated from soils of 'Arhar' fields was undertaken.

The present infrastructure in terms of trained manpower for biotechnological reasearches and other scientific manpower to carry out pulses improvement programmes is woefully inadequate to achieve the target of pulses productivity. During 1993, the government upgraded the Directorate of Pulses Research to the Indian institute of Pulses Research and established three coordinated projects.

Planning for disease control involves strategy and tactics and demands a knowledge of the chemical use for controlling plant diseases. Interest in use of fungicides in plant disease control has vastly increased. In the present study, therefore an attempt has been made to evolve suitable methods for controlling the diseases under investigations.

In present investigation an attempt has been made to study the fungalflora of some unknown and known varieties of pigeonpea. An attempt has also been made to isolate fungi from seeds stored at different length of time in stores. Fungi were isolated, purified, maintained and identified. Pathogenicity tests have been carried out with these organisms. Some bio-chemical studies have also been carried out, which included the effect of some seed-borne fungi on protein contents of seeds of pigeonpea. Effect of environmental factors on disease development has also been observed. In the laboratory fungicides have been evaluated against some pathogenic fungi and the successful ones have been tried in the epiphytotic field conditions. Biological control of *Fusarial* diseases of pigeonpea was also undertaken to find-out an effective biological control method. In the present research work an effort has also been made to investigate the inter-relationship between certain seeds of pigeonpea and their seed borne fungi.

The present problem was undertaken as an integral part of a comprehensive plan of experimental investigation, comploying various organised studies for the furtherance of scientific knowledge in this important and complex fields of research.

Chapter 2
Materials and Methods

Wilt of Arhar (*Cajanus cajan*)
Fusarium equiseti

MATERIALS AND METHODS

"It is common sense to take a method and try it; if it fails, admit it frankly and try another".

— Franklin D. Rossevelt

Seed-borne diseases are commonly observed in pulses and cereals. At the time of seed germination, the resting mycelium or spores lodged inside the seed or seed-coat, become active under suitable conditions, infecting the seedling. According to Wallen (1964), a large proportion of research on seed-borne disease has been mycological in character. As a result hundreds of seed-borne fungi, both pathogenic as well as saprophytic, have been isolated and identified. Various aspects of seed-borne pathogens have been discussed by Noble (1951, 1957) Noble *et al.* (1958) and Dykstra (1961). Host parasite relations and environmental influences in seed borne diseases have been discussed by Wallen (1964). Nene and Agarwal (1978) have given information on some seed-borne diseases and their control. Suryanarayana (1978) gives a comprehensive account of the currently important seed-borne diseases.

Seeds and disease samples of pigeonpea crops from different fields and agencies were collected and fungi were isolated, purified and culture maintained

on Malt-Extract Agar media. A similar isolation study was also carried out with different varieties of pigeonpea crops.

Four techniques viz. Inspection of dry seeds, Seed Washing Test, Standard Blotter Method with or without pretreatment with chlorine and Agar Plate Method with Potato Dextrose Agar medium were used for testing the samples for the presence of mycoflora associated with pigeonpea seeds (ISTA, 1985).

Examination of dry seeds was carried out to see the presence of fungal fructifications such as Sclerotia, Acervuli, Pycnidia, Perithecia etc. and symptoms like discolouration, malformation and spots on its surface by naked eyes and with the help of magnifying hand lens.

To detect the external mycoflora on pigeonpea seeds, fifty randomly selected seeds from each sample were divided into two groups, each was suspended in 10 ml. of sterilized distilled water in the conical flasks, separately. The flasks were shaken by hand for 10 minutes. After shaking, equal volume of this suspension was transferred into two centrifugal tubes. These tubes were kept in centrifuge and rotated at 2000-2500 rpm for 10 minutes. The supernatant liquid was decant-off from each tube and then the sediment from respective tubes was thoroughly mixed in 2.0 ml lactophenol (Booth, 1971) and examined under compound microscope for the presence of fungal spores, fructifications and mycelial fragments.

The viability test of the fungal spores, fructifications and hyphal fragments present in the sediments was carried out by plating the water suspension of the sediment on 2.0 percent water agar in Petridishes. The dishes were incubated at 27 ± 1°C for 5 days and examined under stereoscopic binocular microscope for the presence of fungal colonies, if developed.

Four hundred seeds per sample in four replications, each of one hundred seeds were tested by Standard Blotter Method (ISTA, 1985). Ten randomly selected seeds were plated on three layered moist blotters at equal distance with the help of sterilized forcep in each Petridish (9.0 cm diameter). These seeds were then incubated at a temperature of 27 ± 1°C under twelve hours alternating cycles of light and darkness for 7 days. The seeds were examined for the presence of mycoflora under stereoscopic binocular microscope.

In another experiment, four hundred seeds from each sample were pretreated by dipping the seeds, separately, in 1.0 percent solution of chlorine for a period of 10 minutes and examined by Standard Blotter Method as described above. At the end of incubation period, the fungal species growing on the seeds were transferred and cultured on Potato Dextrose Agar medium and purified by Single Spore Isolation and Hyphal Tip Isolation techniques. The pure cultures, thus obtained were stored on PDA in culture-tubes (i.e. slants) in the refrigerator at 8-10°C for further studies.

Four hundred seeds per sample in four replications, each of one hundred seeds were pretreated with 1.0 percent available chlorine as described in Standard Blotter Method and plated on PDA at the rate of 5 seeds per Petridish. The seeds were incubated at 27 ± 1°C for 7 days under twelve hours alternating cycles of light and darkness and examined macro-scopically by naked eyes for the presence of fungal colonies on seeds. Their specific identification was carried out with the help of standard identification manuals under stereoscopic binocular and compound microscopes.

In order to see the internally seed-borne fungi of pigeonpea, four hundred seeds in four replications, each of one hundred seeds were surface sterilized in 0.1 percent solution of mercuric chloride for 3 minutes. The seeds were subsequently washed in sterilized distilled water for two to three times in order to remove the traces of mercury on seed surface and tested by Standard Blotter Method for the presence of fungi as described earlier.

In order to know the exact location of fungi in the seeds, Component Plate Technique was employed. One hundred seeds, each of UPAS 120 and Pant A 8508 were soaked in sterilized water, individually for over a night and each seed was dissected aseptically into components, i.e. seed coat, cotyledons and ambryonal axis under stereoscopic binocular microscope. These components were surface disinfected in 1.0 percent solution of chlorine for 10 minutes in order to eliminate the surface contaminants. All the components of each seed were then plated

directly on three-layered moist blotters in a Petridish. The seeds were incubated at 27 ± 1°C for 7 days under twelve hours alternating cycles of light and darkness and examined under stereoscopic binocular microscope for the fungal infection in different components.

The growth habit characters of each fungal species as it appeared and looked on pigeonpea seeds were recorded under stereoscopic binocular microscope (6.4 to 40 x magnifications). The cultural and morphological characters of each fungus were studied individually either on Malt Extract Agar or on Potato Dextrose Agar after 7-10 days of incubation. The morphological characters, i.e. shape, size and colour of the sexual and asexual structures of fungi were recorded and measured by the ocular and stage micrometer under compound microscope. The species of fungus were identified with the help of standard identification manuals or monographs.

Both laboratory and pot experiments were carried out in order to determine the pathogenic capabilities of all the fungal species isolated from pigeonpea seeds. The pathogenic nature of the isolated fungal species was ascertained by conducting pathogenicity test in wide mouth tubes as described earlier by Saksena and Kumar (1962). This experiment was conducted in wide mouth (2.5 cm diameter) glass tubes. A roll of blotting paper of 5.0 cm length closed above and opened below was inserted in each tube in the manner, neither very tight nor loose. The top of the tubes were plugged with absorbent cotton. Five ml. of distilled

water was then added to each tube in such a way that the blotter roll remained half immersed in water for being moist throughout experimentation. These tubes were autoclaved at 1.1 kg/cm^2 pressure for 20 minutes. Ten surface sterilized seeds of pigeonpea var. Pusa 85 which showed less infection during isolations were placed on the top of the blotter roll, individually, in each tube and inoculated simultaneously with 5.0 mm culture disc. of 7 to 10 day - old culture of the each fungal species under aseptic conditions. Surface sterilization was carried out by dipping the seeds in 0.1 percent solution of mercuric chloride for 3 minutes and then subsequently rinsed thrice in sterilized distilled water. For control, identical conditions were made except that only 5.0 mm disc of PDA medium without fungal inoculum was placed instead of fungal disc in the tubes for each case. The observations were recorded daily upto 15 days and effect of each fungal species on seeds and seedlings were noted.

To ascertain the pathogenic capabilities of seed-borne fungi, pot experiments were also conducted by using soil inoculation technique. Pots filled with sterilized soil (autoclaved at 1.1 kg/cm^2 pressure for 2 hours) which were previously washed with 5.0 percent solution of formalin. The inoculum was prepared by growing pure cultures of the fungi on corn-meal sand medium (having 3.0 percent corn-meal (w/w) in 5:1 parts of sand - soil mixture) for 30 days. The inoculum of the fungus was mixed at the rate of 5.0 percent (w/w)in the previously sterilized soil, 15 days before sowing of seeds in pots. In control pots, sterilized

corn-meal sand medium without fungal inoculum was mixed. Apparently healthy and surface sterilized seeds of Pusa 85 were sown at the rate of 5 seeds per pot with four replications in each treatment. The pots were kept in glass house and observed critically for seed germination, seedling emergence and effects produced by each fungus on the seeds and seedlings were also recorded upto 30 days after sowing. The seedlings which showed any type of symptom on shoot and or root were considered as 'diseased' and those seedlings which were killed recorded under 'mortality' whether pre and post emergence. The rotted seeds were dug out and examined for the presence of fungus involved.

In leaf inoculation experiments, pigeonpea plants were raised from sterilized seeds of var. Pusa 85 in earthen pots having sterilized soil. The leaves of 30 days - old plants were inoculated by spraying of spore-cum-mycelial suspension of each fungus with the help of a hand automizer. The suspensions were prepared by scrapping of fungal inoculum of 7 to 10 days - old cultures. The inoculated plants were kept in humid chamber for 48 hours in order to provide sufficient moisture to the fungus for causing infection and then transferred in the open environment. Equal number of leaves were also sprayed with sterilized distilled water in the controls. The plants were observed daily and symptoms produced by inoculated fungi were recorded.

Neergaard (1970) proposed an another modification of blotter method which was later accepted by ISTA (1985). The method consists of plating the

seeds in Petridishes on three-layered blotters moistened in 0.001 percent solution of 2, 4-D (sodium salt of 2, 4-Dichlorophenoxy acetic acid) at the rate of 10 seeds per petridish. These dishes were well wrapped in blotter paper in order to reduce evaporation of the 2, 4-D and incubated for 7 days at 27 ± 1°C under twelve hours alternating cycles of light and darkness. The seeds were examined on 8th day for the presence of mycoflora on seeds.

Muskett and Malone (1941) proposed this method which is a modification of agar plate method with PDA. In this method, Malt Extract Agar medium was used in place of Potato Dextrose Agar and seeds were plated on the medium at the rate of 5 seeds per petridish. These dishes were incubated at 27 ± 1°C under twelve hours alternating cycles of light and darkness for 7 days. After incubation period, the seeds were examined macroscopically and under compound microscope for recording the pathogens associated with pigeonpea seeds.

Under laboratory conditions, two hundred seeds in four replications, each of fifty seeds of Pusa 85 were inoculated with spore-cum-mycelial suspension of different pathogenic fungi, individually in order to see their effect on seed germination. Homogeneous suspensions of each pathogen was prepared from 7 to 10 days old cultures as described earlier. The seeds used for inoculation were surface sterilized with 1.0 percent chlorine for 10 minutes. Such seeds were later taken in sterilized Erlenmeyer flasks and 5 ml suspension of each pathogen was mixed to the seeds. The same quantity of sterilized distilled water was added into

the seeds in controls. The flasks were plugged with sterilized cotton and shaken for 15 minutes to get uniform distribution of inoculum on seed surfaces and kept for 24 hours. On the next day, these inoculated seeds were tested by Between Paper Method (ISTA, 1985) for their germination. This method consists off two germination papers of equal size (46 x 27 cm) jointly soaked in sterilized water were placed over a butter paper (39 x 25.5 cm). Fifty seeds were placed at an equal distance over the germination paper which was subsequently covered by another moistened germination paper of same size, then the germination papers alongwith the butter paper rolled up. The rolled sheets were finally kept at a controlled temperature of 25°C in seed germinator for 10 days. After incubation period, these blotter sheets unrolled and the germination percentage of seeds was recorded.

A pot culture experiment was also conducted simultaneously in order to test their effect on seed germination and plant stand under field conditions. One hundred inoculated and uninoculated seeds were sown in pots earlier filled with sterilized soil at the rate of 25 seeds per pot. The observations on the seed germination and plant stand were recorded after 10 and 30 days of sowing, respectively.

The effect of seed-borne fungal pathogens on commercial quality of seeds was studied by the method developed by Srivastava & Tandon (1971). Twenty grams mechanically injured and uninjured seeds of pigeonpea var. Pusa 85 were

put in Erlenmayer flasks of 100 ml capacity after surface sterilization with 0.1 percent mercuric chloride for 3 minutes. The traces of mercury from seed surfaces was removed by repeated 2 to 3 subsequent washings in sterilized distilled water. Surface sterilization was done to eliminate the surface fungal flora of seeds, if any. These seeds were inoculated with each test pathogenic fungus in flasks with three replications. In the control the same quantity of PDA medium was added. These inoculated and uninoculated (control) flasks were kept at room temperature of 20-30°C after plugging with sterilized cotton. The cotton plug of each flask was moistened to provide sufficient humidity to seeds. Observations were critically taken upto 30 days of incubation at a regular interval of 3 days and the effects produced by different pathogens were recorded in relation to discolouration, type of lesions produced, mouldy appearance and extent of seed rotting etc.

Twenty grams of healthy seeds of pigeonpea (variety Pusa 85) were surface sterilized with 0.1 percent mercuric chloride for 3 minutes and then washed repeatedly in 2 to 3 subsequent washings in sterilized distilled water in order to remove the traces of mercury from their surfaces. After surface sterilization, the seeds divided into two lots, each of ten grams and kept in sterilized conical flasks. One out of two lots was inoculated with one ml spore-cum-mycelial suspension, prepared in sterilized distilled water from 7 to 10 day-old cultures of the pathogen and the seeds of the other lot (controls) were maintained by adding one ml of sterilized distilled water instead of fungal suspension. Both the seed-lots were

incubated at 27 ± 1°C for a maximum period of 15 days. Three replicates of each sample were maintained. At the end of 5, 10 and 15 days of storage, the seeds were analysed.

After test incubation periods, the seeds from inoculated and uninoculated seed-lots were dried separately at 65°C in hot air oven for 48 hours and were made in the form of flour by grinding in willey mill. The flour was sieved through a sieve of 50 mesh per square cm. The prepared samples were analysed for protein content, reducing and non-reducing sugars. The total nitrogen was determined by the usual Microjeldahl method and the value thus obtained was multiplied by the factor 6.25 to obtain the protein content (Jackson, 1973), reducing and non-reducing sugars were estimated by potassium ferric cyanide method (AQAC, 1970).

The associative and antagonistic effect between seed-mycoflora of pigeonpea was studied by the method developed by Neergaard, (1970). Two percent PDA medium was used to study the different types of interactions between saprophytes and pathogens and also between pathogens occurring simultaneously on pigeonpea seeds. These fungi made in pairs of all possible combinations. The Petridishes containing PDA medium were inoculated with culture discs (0.5 cm diameter) from 7 to 10 day - old cultures of different fungi for each combination. The pairs were placed at a distance of 2-3 mm apart and incubated at a controlled temperature of 27 ± 1°C in an incubator and watched daily for growth of fungi

involved. After 7 days of incubation, type of interactions between the partners in each pair were recorded.

Eleven systematic and non-systematic fungicides belonging to different groups in their respective doses were evaluated as dry seed dresser for their efficacy in controlling the pathogens associated with pigeon pea seeds and increasing seed germination, seedling emergence and vigour.

Fifty grams of pigeon pea seeds (varieties UPAS-120 and Pant A 8508) were taken randomly in Erlenmayer flasks of one hundred ml capacity and the required quantity of each fungicide was mixed thoroughly with them, separately. The flasks were plugged with cotton and shaked in such a way so that the seeds may get the uniform distribution of fungicide on their surfaces. Two hundred seeds from each treated and untreated seed lots in four replications each of fifty seeds were tested by Standard Blotter Method for finding the efficacy of fungicides in controlling the pathogens associated with seeds.

In another experiment, a total of two hundred treated and untreated seeds of pigeonpea (variety UPAS 120) in four replications, each of fifty seeds were tested by Between Paper Method for their germination under laboratory conditions. The observations on seed germination, shoot length, root length and fresh weight of seedlings were recorded after 10 days of plating of the seeds and analysed statistically. The measurement of shoot length was taken from base to the apical

bud and root length from base to the tip of the root. The average of the shoot and root length was calculated in mm on measurements of ten randomly selected seedlings. The fresh weight of seedlings was recorded in mg and was based on ten seedlings.

These species of *Fusarium* viz., *F. acuminatum* Ellis and Everh., *F. oxysporum* schlecht and *F. solani* (Mart.) Sacc. *F. equiseti* which commonly occur in soils of the Arhar' fields in an around Allahabad were also taken for pathological studies. These studies were carried out from the same stock cultures and hence the method used for isolating, subculturing etc., were Serial Dilution-Agar Plate and Warcup methods. For pathogenicity tests 'Arhar' seedlings of same size and age (4 weeks old) were taken. Pathogenicity tests were carried out by the following methods.

1. The seedling roots both uninjured and injured (10 injuries by sterilized needle per root) were dipped in spore suspension (about 100 spores per lower field of compound microscope) of *Fusarium* species. The seedlings were then replanted in plastic pots. Controls were simultaneously maintained.

2. The seedlings were kept in culture tubes containing 20 days old culture filtrates of *Fusarium* species. In case of control, seedlings were kept in sterilized distilled water. Daily observations were made.

Reisolations were always made in order to confirm the infection with particular *Fusarium* species. Ten seedlings per treatment were taken in each case.

Fusarium species were grown for 20 days in 100 ml of Czapeks Liquid Medium in 500 ml Erlenmeyer flasks. The culture filtrates were collected and centrifuged at 2000 rpm for 20 minutes. The clear supernant was taken and the pH was adjusted to 4.0 by adding 2 NHCl 100 ml of the filtrate was mixed with equal volumes of ethyl acetate atleast for 4 times in a separating funnel allowing 15 minutes for each extraction. All ethyle acetate extract were mixed and evaporated to dryness. 1 to 2 ml of ethanol was added to dissolve the residue. Drops of known volume (0.005 ml) of filtrate as well as an index solution of fusaric acid were kept on Whatman's filter paper no. 1. Spotted choromatogram were runs ascendingly for 10-12 hrs in butanol, formic acid and water (75 : 15 : 10) chromatograms were dried and sprayed by bromophenol blue (.04% in 90% ethyl alcohol). Fusaric acid gives a yellow colour.

In the present study Allahabad University farm soil was selected for survival studies. The soil was dried, and sieved and was infected with 3% maize meal sand culture. This was prepared in 250 ml conical flasks. Each flask was first filled up by 150 g of sand and maize meal mixture 150 g of dry clean sand + 4.5 g of maize meal and 20 ml of distilled water was carefully added (100 gm dry sand holds 20 ml water at saturation so 20 ml from 150 gm sand maize meal mixture gives about 65% saturation) such flasks were then autoclaved for 30 min. at 15 lbs

pressure and were inoculated with agar inoculum disc from a colony margin of 7-10 days old culture of the fungus on Potato Dextrose Agar medium. Flasks were incubated for about 4 weeks at 25 ± 1°C and were shaken after 2 weeks to distribute the fungus.

When the above flasks containing 3% maize meal cultures of *Fusarium* sp. were well grown they were then ready as inoculum for infesting the soil of glass jars. 5 gm of the inoculum (maize meal and culture) was mixed with 100 gm of unsterilized air dried soil to assist in the distribution of inoculum the jar were thoroughly shaken and 20 ml of water was added to give 50% saturation of soil. Such jars were weighed and weight were recorded on the jars. To maintain moisture content at 50% saturation all the jars were weighted on a pan balance about twice a week and distilled water was added carefully until the original weight was recorded. The inoculated glass jars were kept in the laboratory and covered by the petridishes halves to reduce moistures loss. The small gaps served the purpose for air exchange. Five jar per treatment were taken. For the survival studies jars were shaken well before taking out of the soil samples.

Statistical analysis was done whenever consider necessary. The date on percent germination were transferred into angle (ϕ) = $\text{Sin}^{-1}\sqrt{\text{Percentage}}$ before analysis and calculated at 5 percent level of significance by using analysis of variance techniques.

Chapter 3

Isolation and Pathogenicity Tests

Wilt of Arhar (*Cajanus cajan*)
Fusarium acuminatum

ISOLATION AND PATHOGENICITY TESTS

A preliminary diagnosis of a fungal disease is some time possible from the symptoms. Expecting a few such as rust, downey and powdery mildews, which are obligate parasites most plant pathogens grow readily in culture. Before isolating a fungal pathogen from diseased material, the tissue should be examined for fruiting bodies and mycelium. Surface sterilants used for isolating plants pathogens also vary with the nature of the tissue and causative agent.

In the present study a comprehensive survey of seeds and soil samples and infected plant parts from Arhar growing areas of Allahabad, Pratapgarh, Mirzapur, Banda, Fhatehpur, Varanasi and its adjacent areas were done and fungi were isolated.

The method used for soil sampling was similar to that used by Saksena and Mehrotra (1952) and Sarbhoy (1963) samples were taken from all the four sides of pit and at different depths (6", 9" and 12") from all the depths of a locality were mixed together. Soil samples were collected at random, minimum five places from a field. They were packed in sterilized polythene bags and brought to the laboratory. Samples were airdried crushed and passed through 2 mm sieve. The Serial Dilution - Agar planting (or viable plate count method) and warcup method (or direct soil plate method) Warcup (1950) were employed to isolate the various

Fusarium sp. as well as to count the number of *Fusarium* colonies by the use of Quebec colony counter, in a particular sample.

PCNB Agar, modified (Papavizas, 1967) and Potato Dextrose Agar medium were used for culturing soil and seed fungi respectively. The PCNB Agar modified medium containing Agar 20 gm, Peptone 50 gm., KH_2PO_4 1.0 gm, $MgSO_4$.$7H_2O$ 0.5 g., distilled water 1000 ml., Oxgall 1.0 gm, PCNB 0.5 gm., Chlorotetrocyclene HCL 50.0 mg., Streptomycin sulphate 100.0 mg. were added. In Potato Dextrose Agar medium containing Potato tubers (peeled) 200 gm, Agar 17 gm and dextrose 20 gm, water (distilled) 1000 ml.

Taken 500 ml. of distilled water in one litre beaker and added 200 gm washed peeled and sliced potatoes to the beaker. Boil potatoes gentaly for 30 minutes for time till they are easily penetrated by glass rod and then filter through cheese cloth, squeezing out all liquid. Added 20 gm dextrose to the potato extract. Taken 500 ml of water in another beaker and heat it and added 17 gm agar, bit by bit to the hot water (96°C) to dissolve it and make it 1000 ml adding by distilled water. Despense 200 ml each to five conical flask after plugging sterilize at 15 Lbs/inch^2 (121°C) pressure for 15 minutes in an autoclave.

Now a days, 0.85% sodium chloride (NaCl) is being preferred as a diluent, in place of sterilized water for the preparation of suspension of soil sample

employed Serial Dilution-Agar Plate Technique.

Spread 0.1 ml on replicable petri-plate containing appropriate medium.

Incubation of plates at 25°C for 2-7 days is an inverted position.

Count number of colonies of fungi in each plate using Quebec colony counter

Calculated the number of fungi per gm of soil

$$\text{Variable cell/gm dry soil} = \frac{\text{Mean place count} \times \text{Dilution factor}}{\text{dry wt. of soil}}$$

Table : 1

Fungi isolated from seeds of pigeonpea and soils from its cultivated fields of Allahabad District

Regions	Soils	Seeds
Agriculture Farm University of Allahabad		
	Fusarium oxysporum	**Fusarium semitectum*
	F. solani	**Fusarium moniliforme*
	F. equiseti	**Curvularia lunata*
	F. moniliforme	*Microphomina phaseolina*
	Aspergillus flavus	**Alternaria alternata*
	A. niger	**A. longissima*
	Rhizopus stolonifer	**Aspergillus flavus*
	R. sp.	*A. niger*
	Penicillium notatum	*Botrytis cinerea*
	Saprolegnia sp.	*Cephalosporium sp.*
	Mucor mucedo	*Chaetomium globosum*
	M. javanicus	*C. olivaceum*
Chaka Block	*Mucor hiemalis*	*C. setosum*
	M. mucedo	*C. offine*
	M. spinosus	**Cladosporium cladosporiodes*
	M. racemosus	**Colletotrichum dematium*
	Fusarium solani	**Curvularia lunata*
	F. acuminatum	*Drechslera tetramera*
	F. equiseti	*Helminthosporium tetramera*
	F. oxysporum	*Bipolaris tetramera*
	Aspergillus niger	**Fusarium moniliforme*
	A. flavus	**F. semitectum*
	A. nidulance	*Mucor mucedo*
	A. fumigatus	*Mucor sp.*

Bara Region	*Penicillium sp.*	*Pencillium oxalicum*
	Mucor javanicus	*Phoma sp.*
	M. circinelloides	**Phyllosticta cajani*
	M. ramosissimus	**Rhizoctonia bataticola*
	M. spinosus	**Rhizoctonia solani*
	Rhizomucor miehei	*Rhizopus arrhizus*
	Rhizomucor pasillus	*Rhizomucor pasillus*
	Fusarium oxysporum	*R. nigricans*
	Fusarium equiseti	**Trichothecium roseum*
	F. solani	*Phoma emblica*
	Rhizopus equinus	*Alternaria sp.*
	R. cohnii	*Fusarium equiseti*
	R. microsporus	**Aspergillus flavus*
	Aspergillus niger	*A. sogae*
	A. nidulance	*A. wentii*
Karchana Region	*Pilobolus klunii*	*Pencillium notatum*
	Rhizopus arrhizus	*Phytophthora infestans*
	R. cohnii	**Fusarium oxysporum*
	R. microsporus	*Aspergillus nidulance*
	Mucor mucedo	*A. fumigatus*
	M. hiemalis	*Rhizopus arrhizus*
	M. spinosus	*R. microsporus*
	Fusarium semitectum	*Rhizomucor miehei*
	F. equiseti	*R. pasillus*
	F. moniliforme	*Phyllosticta cajani*
	F. roseum	*Rhizoctonia bataticola*
	Aspergillus flavus	*Assochyta phaseolorum*
	Aspergillus niger	*Colletotrichum lindemuthianum*
	Pencillium sp.	**C. dematium*
Koraon Region	*Alternaria alternata*	*Aspergillus niger*
	Phoma emblica	*A. fumigatus*

	Fusarium solani	*Rhizopus eqiunus*
	F. decentcelluelare	*R. cohnii*
	F. oxysporum	*Aspergillus nidulance*
	Aspergillus oryzae	**A. flavus*
	A. sogae	**Fusarium semitectum*
	A. wentii	**F. acuminatum*
	Pencillium notatum	*Drechslera cajani*
	P. sp.	*Botrytis cinerea*
	Mucor mucedo	*Cephalosporium sp.*
Meja Region	*M. javanicus*	*Mucor mucedo*
	Fusarium oxysporum	*M. javanicus*
	F. equiseti	*M. ramosissimus*
	F. acuminatum	**Cladosporium cladosporiodes*
	Alternaria alternata	**Alternaria longissima*
	Alternaria oryzae	**A. alternata*
	Aspergillus niger	*Chaetomium globosum*
	A. phailiseptus	*C. setosum*
	A. clavatus	*Helminthosporium tetramera*
	Pencillium sp.	*Phoma sp.*
	Mucor spinosus	**Rhizoctonia bataticola*
	M. ramosissimus	*Fusarium equiseti*
	M. hiemalis	*Rhizopus stolonifer*
Handia Region	*Fusarium oxysporum*	*R. arrhizus*
	F. semitectum	*R. nigricans*
	F. solani	*Rhizomucor passilus*
	F. equiseti	**Rhizoctonia solani*
	Mucor mucedo	**Alternaria alternata*
	M. spinosus	*Aspergillus nidulance*
	Rhizopus stolonifer	**A. flavus*
	R. arrhizus	*Macrophomina phaseolina*
	Rhizomucor miehei	*Rhizoctonia solani*

Phoolpur Region	*Macrophomina phaseolina*	*Rhizopus microsporus*
	Curvularia lunata	*Botrytis cinerea*
	Aspergillus flavus	**Trichothecium roseum*
	A. oryzae	*Phoma emblica*
	A. fumigatus	*Aspergillus flavus*
	A. spinulosum	*A. wentii*
	Rhizopus stolonifer	*Ascochyla phaseolorum*
	R. arrhizus	**Colletotrichum dematum*
	Rhizomucor miehei	*Bipolaris tetramera*
	F. oxysporum	*Cephalosporium sp.*
	F. solani	**Alternaria longissima*
	F. equiseti	*Mucor mucedo*
	F. semitectum	*M. javanicus*
	Mucor spinosus	*M. ramosissimus*
	M. ramosissimus	*Rhizomucor miehei*
Soraon Region	*Fusarium oxysporum*	*Cladosporium oxysporum*
	F. solani	**Fusarium decentcelluelare*
	F. equiseti	**Fusarium roseum*
	F. acuminatum	**F. semitectum*
	Phytophthora infestans	*Colletotrichum lindemuthianum*
	Penicillium notatum	**Cladosporium cladosporiodes*
	P. olavigerum	*Mucor hiemalis*
	P. sp.	*M. racemosus*
	Talaromyces vermiculotus	*M. mucedo*
	Rhizopus equinus	*Phyllosticta cajani*
	R. arrhizus	*Drechslera tetramera*
	Rhizopus microsporus	**Fusarium oxysporum*
	Aspergillus flavus	**F. acuminatum*
	Aspergillus niger	*Rhizopus nigricans*
	Mucor mucedo	*R. arrhizus*

	M. racimosus	*Rhizomucor pasillus*
	Alternaria alternata	*Phoma sp.*
Begum Sarai	*Fusarium solani*	**Curvularia lunata*
	F. oxysporum	*Ascochyta phaseolorum*
	F. moniliforme	*Macrophomina phaseolina*
	F. semitectum	*Aspergillus niger*
	Mucor spinosus	*A. nidulance*
	M. ramosissimus	*A. fumigatus*
	M. mucedo	*Penicillium notatum*
	Aspergillus flavus	**Fusarium moniliforme*
	A. niger	**Trichothecium roseum*
	A. phailiseptus	**Colletotrichum dematum*
	A. clavatus	*Chaetomium globosum*
	Rhizopus stolonifer	*C. affine*
	R. arrhizus	*Cephalosporium sp.*
	Rhizomucor miehei	**Alternaria alternata*
Chandra Shekhar Azad Park	*Alternaria alternata*	**A. longissima*
	F. solani	*Cladosporium oxysporum*
	F. semitectum	*Macrophomina phaseolina*
	Aspergillus flavus	**Rhizoctonia solani*
	A. niger	**Trichothecium roseum*
	A. nidulance	*Mucor spinosus*
	A. fumigatus	*M. mucedo*
	Mucor spinosus	*Aspergillus clavatus*
	M. mucedo	*A. phailiseptus*
	M. racimosus	*Pencillium sp.*
	Rhizopus stolonifer	*P. notatum*
	R. arrhizus	**F. oxysporum*
	R. sp.	**F. semitectum*
	Rhizomucor miehei	*F. equiseti*

Khushroo Bagh	*Aspergillus flavus*	**F. moniliforme*
	A. nidulance	*Rhizopus stolonifer*
	A. fumigatus	*R. arrhizus*
	A. phailiseptus	*Botrytis cenaria*
	A. clavatus	*Phytophthora infestans*
	Fusarium oxysporum	*Phoma sp.*
	Fusarium solani	**Rhizoctonia bataticola*
	F. semitectum	*Phyllosticta cajani*
	F. acuminatum	*Macrophomina phaseolina*
	F. equiseti	**Cladosporium cladosporiodes*
	F. moniliforme	*Cladosporium oxysporum*
	Mucor mecedo	**Trichothecium roseum*
	M. spinosus	**Fusarium decentcelluelare*
	M. hiemalis	**F. roseum*
	Rhizopus stolonifer	*Mucor mucedo*
	R. arrhizus	*M. hiemalis*
	Rhizomucor miehei	*M. racemosses*
	Alternaria alternata	**Fusarium moniliforme*
	Alternaria oryzae	**F. semitectum*
	Phoma emblica	**F. acuminatum*
	Penicillium sp.	*F. equiseti*
		F. solani
		Ascochyta phaseolorum
		**Curvularia lunata*
		**Alternaria longissima*

* Denotes internally seed-borne

Seeds of local varieties were collected from different regions of Allahabad, surface sterilized with 0.1% murcuric chlorid solution and tested by Standard Blotter Method and Component Plate Technique for externally and internally seed-borne pathogens. Fungi isolated and identified were listed in Table 1. Result show that fifty eight species of twnety three genera were present, in which sixteen species of eight genera found internally seed-borne consequently remaining fourty two species of fifteen genera were externally seed-borne. They were single genera of Mastigomycotina, three genera of Zygomycotina, six genera of Ascomycotina and thirteen genera of Deuteromycotina subdivision.

Fungi isolated from soil samples by the used Serial Dilution-Agar Plate Technique and Warcup method culture maintained and identified, list in table 1. In soil samples fourty three species of fourteen genera were found in which only two genera of Mastigomycotina, four genera of Zygomycotina, three genera of Ascomycotina and five genera of Deuteromycotina subdivision.

Table : 2

Varietal samples of pigeonpea collected from different places and date of collection

S.No.	Variety	Seed Stokists/Agencies	Date of Collection
1.	Bahar	N. D. University of Agric. & Tech. Narendra Nagar, Kumarganj, Faizabad (U.P.)	11-11-2000
2.	GAUT 82-58	-- do --	-- do --
3.	GAUT 82-90	-- do --	-- do --
4.	Pant A-8505	-- do --	-- do --
5.	Pant A-8508	-- do --	-- do --
6.	ICPL 151	Directorate of Pulses Research (ICAR) Kanpur	05-12-2000
7.	ICPL 317	-- do --	-- do --
8.	ICPL 83006	-- do --	-- do --
9.	ICPL 83015	-- do --	-- do --
10.	C 11	Division of Mycology and Plant Pathology IARI New Delhi	10-12-2001
11.	AL 15	-- do --	-- do --
12.	Prabhat	Legume Section, C.S.A. Univ. of Agric. & Tech., Kanpur	25-01-2001
13.	Pusa 18	Directorate of Pulses Research (ICAR), Kanpur	27-01-2001
14.	Pusa 85	-- do --	-- do --
15.	Pusa 604	-- do --	-- do --
16.	Pusa 851	-- do --	-- do --
17.	T 7	-- do --	25-01-2001
18.	T 17	-- do --	-- do --
19.	T 21	-- do --	-- do --
20.	UPAS 120	U.P. Seeds and Tarai Development Corporation, Regional Station, Kalyanpur, Kanpur	17-04-2001

The varietal samples collected from different Agriculture Universities and Agencies were listed in Table 2. Surface sterilization, Standard Blotter method and Component Plate Technique were used and examined under compound microscope as described in 'Materials and Methods'. The examination of suspensions showed presence of spores of *Alternaria, Aspergillus, Curvularia, Drechslera* and *Fusarium* under compound microscope. Spores of *Alternaria alternata* were prevalent in the washings of the seeds of all the varieties except Pusa 85. Aspergillus were the next in predominance having found in the washings of seeds of almost all the varieties except **C 11, ICPL 151, ICPL 317, Pant A 8505, Pant A 8508, Pusa 85 and Pusa 851**. Varieties UPAS 120, Prabhat, T 7, T 21 and Bahar exhibited the presence of spores of *Curvularia lunata* in their washing. *Drechslera tetramera* spores were recorded in T 21, ICPL 83006 and UPAS 120. Macroconidia of *Fusarium* species were also observed in the most of the varieties.

The seeds of pigeonpea cultivar UPAS 120 which showed maximum number of fungal species on blotters, were separated into three categories, viz. apparently healthy looking, discoloured and shrivelled seeds as described earlier. Two hundred seeds of each category were tested in four replications, each of fifty seeds. The observations of the fungi present on the seeds were taken after 7 days of incubation.

Earlier workers have indicated that seed-brone fungi caused discolouration and shrivelling of pigeon pea (Kumar and Patnaik, 1985). In order to determine the fungi associated with different categories of seeds, variety UPAS 120 was divided into three categories and tested by Standared Blotter Method as described in `Materials and Methods'. The observations were taken after 7 days incubation and are presented in table 3.

The results of table 3 indicate that normal sized apparently healthy looking seeds of pigeon pea variety UPAS 120 carried less number of fungal species as compared to discoloured and shrivelled seeds. The number of fungal species on under sized and on normal sized discoloured seeds were fourteen and eighteen, respectively, while shrivelled of both the sizes yielded nineteen fungal species. Discoloured seeds showed association of almost all fungal species except *Mucor* sp. predominantly being *A. alternata, A. flavus, P. cajani* and *R. bataticola*. Undersized shrivelled seeds were found most heavily infected as they yielded more colonies of almost all the fungal species than that on normal sized seeds. The frequency of *A. alteranta, A. flavus, A. niger, F. moniliforme, F. semitectum, P. cajani, R. bataticola* and *F. solani* was relatively high in normal sized seeds and even more high in undersized shrivelled seeds. Under sized apparently healthy looking seeds carried only eleven fungi that too in less number. Colonies of *B. cinerea* and *C. lunata* were not recorded on normal sized apparently healthy looking seeds. Some fungal species, viz. *A. alternata, A. flavus. A. niger, B.*

cinerea, C. dematium, F. moniliforme, F. semitectum, categories and grades of pigeon pea seeds.

Fungi associated with seeds may be carried externally on seed surface or internally inside the seeds. To determine its, the seeds of varieties UPAS 120 and pant A 8508 were surface sterilized with 0.1 percent mercuric chloride solution and tested by Standard Blotter Method as described in `Materials and Methods'. The recovery of fungi from internal tissues of such seeds were recorded after 7 days of incubation and are listed in table 4.

The results of Table 4 reveal that out of twenty-two fungal species found associated with unsterilized seeds of pigeonpea, only twelve fungi, viz. *A. alternata, A. longissima, A. flavus, C. cladosporioides, C. dematium, C. lunata, F. moniliforme, F. semitectum, P. cajani, R. bataticola, R. solani* and *T. roseum* were recovered from surface disinfected seeds. The number of colonies of each fungus was also less than that of untreated seeds. It indicates that these fungi survive inside the tissues of seeds and remaining fungi seemed to be associated externally.

Seed- borne pathogens may be located superfecially or internally in the seed. The knowledge of exact location of the fungus in seed is helpful in devicing control measures. Component Plating Technique was employed as described under `Materials and Methods' for ascertaining the location of pathogens. One hundred seeds of varieties UPAS 120 and pant A 8508 each were used as the former variety

carried all the pathogens except *Alternaria longissima* which was only recorded on pant A 8508. The observations are summarized in Table 5.

The results of Table 5 show that all the twelve fungi as observed on mercuric cholride seeds were found to be carried on the seed coat of all the infected seeds except *C. dematium, C. lunata* and *F. semitectum*,. The later fungi was detected from 66.7, 75.0 and 80.0 per cent of the infected seeds, respectively, Infection of *A. flavus, C.dematium, F. moniliforme, F. semitectum, P. cajani, R. bataticola* and *R. solani* was observed in the 28.5 to 60.0 per cent cotyledons of the infected seeds, indicating that these can move inside the seed of become internally seed-borne. The tissues of embryonal axis yielded the colonies of *F. moniliforme, P. cajani, R. bataticola* and *R. solani* in 16.7 to 28.5 per cent of the infected seeds.

Growth habit characters of each fungal species as it appeared and looked on the seeds, were recorded under stereo-scopic binocular microscope (6.4 to 40.0 x magnification). The cultural and morphological characters of each fungus were studies individually after 7 to 10 days of incubation under compound microscope as described under `Materials and Methods'. The characters of each fungus is described below:

Alternaria alternata **(Fr.) Keissler**

Syn. *A. tenuis* Nees ex Pers.

Long chains of dark brown to blackish spores alongwith scanty greyish to brown mycelium could be seen on all over the seed. In most cases, the fungus grew in association with other fungi. Seedlings showing growth of *A. alternata*, sometime, exhibited brown rot symptoms on its hypocotyl.

Colonies on PDA were fast growing, usually black or olivaceous black in later stages, sporulation abundant; Reverse of the colonies olivaceous black; Mycelium septate, branched, olive-brown, 2.0-9.0 μ thick, sometimes swollen (10.5-21.0 μ) to form chains of chlamydospores; conidiophores arise singly or in groups, usually simple or branched, straight or flexuous, septate, golden brown to pale or olivaceous, geniculated, bear conidia, variable in length 23.0-45.5 μ and 2.5-4.5 μ thick; conidia formed in long simple or branched chains, golden brown in colour, usually smooth walled, sometimes rough walled, obvoid to obclavate, muriform with up to 7 transverse septa (av.4-5) and 0-4 μ Longitudinal septa, 12.0-55.0 μ (av.34.2) x 5.0- 14.0 μ (av. 11.5) including beak. Beak usually 2.0-11.0 μ (av.8.0) x 2.0- 4.2 μ thick, not more than one third of conidial length. Branching of chains normally takes place at the beak of a spore which show several scars and become geniculate, sometimes the branch of chains arises from a short lateral projection on the beak; chlamydospores not observed.

Alternaria longissima **Deighton and MacGarvie**

Brown conidial mass with scanty aerial mycelium was seen on whole seed surface. Conidia were borne singly on a very long straw coloured conidiophores, variable in shape but commonly filiform (obclavate to rostrate), *Cercospora* like long, appearing under stereoscopic binocular microscope as hyphal branches.

Dull white to light brown, fluffy and cottony colonies of the fungus developed on PDA; Reverse of the colony was pale-olivaceous; Mycelium partly superfecial and immersed; conidiophores arise singly, errect or ascending, simple or occasionally branched, straight or slightly flexuous, sometimes geniculate, somewhat swollen at the apex, sub-hyaline or pale to mid-pale brown, smooth below, varruculose at and sometimes below the apex and measured upto 165 μ long and 3.0-5.0 μ thick with one to several conodial scars and 2-15 septate; conidia solitary or occasionally in chains acropleurogenous, pale straw to brown coloured, highly variable in shape but commonly obclavate to rostrate, Cerecospora-like obclavate with basal subcylindric portion of few cells and hypha like long, upto 85-498 (av.342) μ, narrow, septate, beak having 6.0-34.0 (av.24.5) μ transverse septa and realy few oblique septa, 5.0-13.0 (av.8.0) μ thick in the broadest part and 2.0-3.0 (av. 2.5) μ thick at the apex; Chlamydospores not observed.

Aspergillus flavus **Link**

Compact, spherical or columber, yellowish-green, large, globose to dome-shaped radiate, conidial heads of the fungus with very little mycelium was observed on seeds.

Colonies on MEA fluccose, fast growing, white in the begining and later developed in different shade of green or shamrock-green to yellowish green; Reverse of the colonies yellowish and later turn into brown shades with age; conidial heads large, yellowish-green, globose to dome-shaped, radiate, 27.0-90.8 (av.57.5) μ in diameter; Conidiophores colourless, rough walled, 419.6-1562 (av. 973.0)x 6.4-10.0 (av.8.5) μ ; Vesicle dome-like to flask shaped, 21.0- 33.5 (av. 27.5) μ in diameter; Sterigmata in one series in small heads, measured 7.2-11.0 x 2.5-4.8 μ or in two series in larger heads, primary sterigmata 4.4-9.5 x 2.2-3.0 μ and secondary 6.4-9.5 x 2.2- 3.0 μ; conidia almost globose, echinulate, colourless to yellowish- green and 2.8- 4.5 μ in diameter; Sclerotia and Perithecia not observed.

Aspergillus niger **van Tiegh.**

Dark brown to black but almost black, globose, mostly radiate but columner masses of conidial chains developed on seed in clusters or singly form a foot cell; spore mass appeared on conidial heads.

Colonies on MEA fast growing with abundant submerged mycelium and aerial hyphae scanty and blackish-brown to carbonous-black; Reverse of the colonies pale olive-buff; Conidial heads blackish-brown to purple-brown, globose, radiate, 54.0-226.4 μ in diameter; Conidiophores often arise directly from substratum, usually light-brown near vesicle, remaining portion colourless, smooth, aseptate, varying in length 228.5-720.5 x 14.0-22.5 μ, thick walled; Vesicle globose, 24.5-61.0 μ in diameter; Sterigmata yellowish-brown typically in two series, primary sterigmata more or less wedge shaped closely packed and measured 13.6-21.8 x 2.2-3.0 μ and secondary sterigmata bottle shaped, 6.4-9.0 x 1.2-2.2 μ in size; Conidia globose, at first smooth, later spinulose with colouring substance, wall blackish-brown, almost blackish-brown in mass, 3.2-5.0 μ in diameter; Sclerotia and Perithecia not observed.

***Botrytis cinerea* Pers. ex Fr.**

White a grey colonies were observed around the affected seeds which containing strands of brown, septate, branched hyphae; under stereoscopic binocular microscope, the branches have constriction at their point of origin. At the tip and at intervals along the hyphae, clusters of conidia occur. They are formed on small pegs on the swollen ends of hyaline branches.

Colonies on MEA grew rapidly forming a rather thin white colony which later turns from dark olive-green to brown-black in colour, when it comes into

contact with the glass of Petridish, it forms attachment organs, sporulation takes place after 2 weeks; Mycelium septate, brown, young hyphae thin and hayline, 7.0-17.2 μ thick; Conidiophores errect, septate, 8.0-22.8 μ thick, tip of conidiophores or its branches slightly enlarge and bear small pointed sterigmata, light-brown in colour with hayline tips; Conidia hyaline, single celled, oval, globose or short cylindrical, borne in clusters at the tip of the hyphal branches and measured 6.0-14.6 (av. 8.4) x 6.0-9.5 (av. 7.0) μ, germinated by producing 1-3 thin hyaline germtubes; Sclerotia not observed.

***Cephalosporium sp.* Corda**

Numerous simple or occasionally branched, hyaline, aseptate, conidiophores bearing cylindrical or curved or occasionally avoid conidia with scanty mycelium were observed on seeds.

Colonies on MEA grew slowly, forming a rather dense white colony which become tingled with seldom pink; Reverse of the colony also seldom pink coloured; Mycelium (vegetative hyphae) cobwebby, sparsely septate, branched, hyaline, 1.0-1.5 μ thick, frequently aggregating to formed threads; Conidiophores are formed numerously at right angle to the hyphae, simple or occasionally branched, hyaline, aseptate, 15.4-30.0 μ long and 1.0-1.5 μ thick at the base, tapering towards the tip where conidia are borne in a globules of liquid; Conidia are borne single, hyaline, continuous, cylindrical, with rounded ends, sometimes slightly curved, occasionally ovoid, 3.0-6.5 x 1.0-2.0 μ.

***Chaetomium globosum* Kunze ex Fr.**

Syn. *C. olivaceum* Cooke and Ellis
C. setosum Bainier
C. affine Corda

Separate, dark with greenish-brown or grey hairs, sub-globose or ellipsoidal perithecia were observed on seeds.

Perithecia fastened on MEA by dark rhizoids, scattered or gregarious, ovate to ellipsoid, often pointed at the base and measured 200.0-250.0 x 185.0-250.0 (av. 217.0 x 208.0) μ with hairs, i.e. lateral hairs are flexuous, brown, sparingly septate, finely roughened, 2.0-3.0 μ thick and terminal hairs numberous, undulate or more frequently coiled, brown, finely roughened, aseptate, 2.0-4.0 μ thick; Asci clavate, slightly apiculate at both ends, the fertile portion measured 24.0-30.2 x 10.5-15.8 μ and containing 8 spores; Ascospores brown, sub-spherical to ellipsoidal with umbonate ends, 8.2-10.5 x 6.6-8.0 μ.

***Cladosporium cladosporioides* (Fr.) de Vries**

Conidial heads composed of large number of smooth-walled spores, a small proportion of which are larger than the remainder. Colonies frequently grew on seeds having conidiophores and conidia and aerial mycelium rather scanty.

Colonies on MEA usually dark green, velvety and relatively slow growing, their production of aerial mycelium rather scanty but normally sporulate freely, the

conidia being formed in chains; Reverse of the colony dark coloured; Mycelium hyaline, becoming dark, septate, smooth or finely roughened, 2.0-3.5 μ thick; Conidiophores arise laterally from the mycelium or formed terminally on the hyphae, brown, smooth or finely roughened, septate, upto 85.0 μ long and 3.0-4.0 μ thick at the base, tapering slightly towards the tips; Conidial heads composed of chains of spores of varying in size, a large proportion being of small conidia and a small proportion of large conidia; Conidia borne in chains, brown, viable in shape, continuous, occasionally 1 or 2 septate, not obviously rough walled, sometimes tending to become cylindrical, pointed towards the base and irregular at the distel end due to protrusions on which conidia formed and measured 3.5-16.0 x 2.0-4.0μ.

***Colletotrichum dematium* (Fr.) Grove**

Acervuli single or in groups, setae numerous, blackish brown to dark black, longer than the conidial mass. Conidial mass white to dull white, pale orange or bright orange. Mycelium mostly absent when present fine, shiny or whitish pink.

Colony on PDA compact, creamy in colour in initial stage which later on turn blackish brown to dark brown; Reverse of the colony black; Mycelium slender, septate when young hyaline and contains droplet of oil globules and later become brownish in colour and 3.0-5.0 μ in thickness; Acruvuli hemispherical, the immature acervuli greyish-white to dull orange and mature dark brown in colour; Setae numerous, trichiform, brown to blackish brown, 2-7 septate, 71.0-215.0 x

3.0-5.0 μ and varied in number from 2-15 per acervullus; Conidiophores arranged in definite layers on the surface of stomatic tissues, short, errect, simple, hyaline, aseptate and packaged together and measured 12.5-20.5 x 3.0-4.5 μ; Conidia hyaline when single but white to dull white or pale orange in mass, fusoid, ends rounded or slightly tapering, 19.0-25.5 x 3.0-5.0 μ in size.

***Curvularia lunata* Boedijn**

Pale olivaceous to black colonies of the fungus was seen on the seed surface from which conidiophores arise singly and bearing pale olivaceous to black coloured conidia of mostly curved and straight also, acropleurogenously under stereoscopic binocular microscope.

Colonies on PDA fast growing, appreassed, smooth, at first white becoming dark, greenish olive to black in colour due to copious sporulation. Reserve of the colony olivaceous black and smooth; Mycelium septate, branched, hyaline later turns brown, 3.0 - 5.6 μ in thickness; Conidiophores arise singly, mostly simple or sometimes branched, geniculate at tips, brown, 16.0-102.8 x 3.2-5.0 μ; Conidia one to five septate but mostly three septate, olivaceous black to dark brown, third cell from the base usually larger in size and darker in colour than other cells, end cells subhyaline or pale brown, curved mostly, when straight the apical cell rounded, constriction at septa not prominent and measured 18.5-32.0 x 7.0 - 15.0 (av. 27.5 x 9.0) μ; Chlamydospores not observed.

Drechslera tetramera **(Mc Kinney) Subram. & Jain**

Light to dark brown, ellipsoid to almost cylindric with rounded ends, typically 4 celled conidia were formed on the brownish conidiophres at short intervals in clusters under stereoscopic binocular microscope. Conidiophores arise singly or in clusters of 2 to 3 and bearing conidia acropleurogenously.

Colonies on PDA fast growing, compact subfloccose becoming felty and plane, later on, quaker drab to dark greyish olive in colour; Reverse pale-olive to light brown; Mycelium septate, branched, dark olivaceous and 3.2-5.0 μ thick; Conidiophores usually simple, septate, brown, straight at the base but geniculate irregularly at tips and bearing conidia acropleurogenously at the tip and measured 44.2-178.0 x 6.0 - 8.4 μ; Conidia mostly 4 celled, brown, ellipsoid and mostly cylindric, straight with broad rounded ends, lighter towards terminal cells, 18.0 - 35.6 x 6.5 - 11.8 μ in size.

Fusarium moniliforme **Sheldon**

Colonies on seeds were whitish or purple in colour with easily recongizable with white chains of microconidia and scanty mycelium. Small heads of microconidia on short and simple sterigmata were also observed which gave an appearance of powdery form.

Colonies on PDA fast growing, smooth, white, compact, appearing quite powdery with the production of large number of microconidia; Mycelium septate,

branched white or purplish white or very little orange white, varying from 1.5-4.5 μ in thickness; Microconidia produced in long chains dominating over macroconidia, small, one or 2 celled, hyaline, egg-shaped, elliptical to ovate, 4.2-16.0 x 2.5-5.2 μ; Macroconidia thin walled, scattered, formed in sporodochia or pinnotes, subulate to cylindrical, slightly curved, narrowing at both the ends, browinish white in mass, one to 5 septate but mostly 3 septate, and measured on the basis of septation.

No. of septa	Size of macroconidia
1	14.5-22.0 x 3.2-4.8 μ
3	26.0-38.8 x 4.0 - 5.5 μ
5	32.2-45.6 x 4.5-6.0 μ

Chlamydospores not observed.

***Fusarium semitectum* Berk. & Rav.**

Whitish or light orange white, compact mycelial growth covering the whole seed was seen alongwith the characteristic whitish, shiny, highly branched conidiophores bearing easily recognizable macroconidia giving an appearance of 'flower'. Branched conidiophores with macroconidia easily be observed on the periphery of colonies.

Colonies on PDA whitish incarnate or isaballin with abundant aerial mycelium of whitish cottony growth in the early stages and later changed into light yellow or buff brown; Mycelium septate, 3.0-6.0 μ thick, brand creeping; Conidiophores arise singly or in groups, whitish, shiny, highly branched bearing

macroconidia; Macroconidia developed on aerial mycelium as fusoid, hyaline, 0 to 5 septate but occasionally upto 7 septate, wedgeshaped, measured on the basis of septation.

No. of septa	Size of macroconidia
0	4.0-16.0 x 2.0-4.0 μ
1	8.0-20.0 x 2.0-4.5 μ
3	23.0-50.0 x 3.2-6.2 μ
5	32.0-65.0 x 4.0-6.5 μ
7	37.0-77.0 x 4.2-6.5 μ

Microconidia and Chlamydospores not observed.

***Mucor* sp. Mich. ex Fr.**

Mucor sp. was readily identified by the presence of spherical sporangia containing numerous spores and absence of rhizoids. The sporangia which when removed reveal a swelling at the tip of the sporangiophore - the columella.

Colonies on MEA grew rapidly, variable in shape of white or cottony, later on changing in greyish - yellow with time; Reserve of the colony buff yellow. A well developed colony reach to the lid of the Petridish with much aerial mycelium as white, grey or brown in colour in which sporangia were normally produced free; Sporengiophores usually unbranched errect, turf abut 10.0 mm high or upto 14.0 mm high; Sporangia spherical to globose, grey or brownish yellow, bright yellow with age, visible to the naked eyes, 24.2-78.0 (av. 51.5) μ in diameter;

Columella free, spherical or oval, colourless, measured 13.2-32.0 (av. 27.8) μ in diameter and oval 20.0-32.0 (av. 27.8) x 16.5-30.2 (av. 22.5) μ; Spores unequal, mostly elongate, ellipsoid or kidney shaped, 3.2-7.0 x 1.8-5.5 μ in size; Zygospores and Chlamydospores not observed.

***Penicillium oxalicum* Currie & Thom.**

Usually greenish coloured spore mass of the fungus was observed on all over the surface of seeds which consist of conidiophores and conidia. Spore heads were broom-like bearing chains of spores.

Colonies on MEA slow to moderate rate of growth, fluccose, in shades of blue green, dusky green to dark yellowish green; Reverse of the colony ivy to colonial buff; Mycelium usually not very plentiful but sporulation takes place freely giving a colony which is usually some shades of green; Spores produced in a broom like penicillus; Penicillii typically biverticellate and asymmetric, light yellowish green, 15.5-21.0 x 4.8-11.2 μ in size, borne on smooth walled conidiophores arising directly from the substratum; Conidiophores almost hyaline, sparsely septate, bearing metulae often in groups and terminal clusters of 5 to 6, measured 88.2-160.5 x 3.0 - 5.5 μ Conidial heads columner, usually upto 214.0 μ long; Metulae usually in groups or in terminal clusters of 5-6, measured 7.5-12.5 x 2.5-3.5 μ ; Conidia ellipsoidal, smooth, light yellowish green or pale green in mass, 3.0-55 x 2.2-3.5 μ in size; Sclerotia and Cleistothecia not observed.

Phoma **sp. Desm.**

Smoky grey to light grey colonies of the fungus containing large number of dark brown pycnidia scattered on all over the seed surface. Pycnidia were sub-epidermal, oval, ostiolate, mostly not oozing out.

Colonies on MEA moderately rapid and easily seen by naked eyes after 5-6 days which being a shade of smoky grey, tend to lighten the colour; Reverse of the colony intensive dark chocolate brown to almost black; Mycelium superfecial, dark grey brown to almost black in colour with a substantial development; Pycnidia sub-epidermal, dark brown, sometimes crowded, thin walled each with a single chamber and generally having one ostiole, size very variable from 95.0-260.0 x 78.0-260.0 μ, ostiole circular to subcircular, 5.5-14.0 μ in diameter; Conidiophores simple, hyaline, somewhat tapering above, 8.2-21.0 x 1.2-2.5 μ; Conidia single celled, elliptical, hyaline, normally bi-guttulate, 5.5-9.0 x 2.5-4.5 μ, extruded from the ostiole of the pycnidium in the form of creamy coloured, curved tendril or few of them two celled; Chlamydospores not observed.

Phyllosticta cajani **sydow**

Light brown to black pycnidia with or without beak were generally observed on seed surface. Only on few infected seeds, white scanty mycelium consisting of hyphae was seen near pycnidia. Pycnidia were usually solitary, scattered on all over the seed surface but sometimes in clusters in the hilum region. Ooze of pycniospores was at times observed from some pycnidia.

Colonies on PDA compact, cottony with profuse mycelial growth and zonations of different shades of green; Mycelium hyaline, later turn dark brown in colour, aseptate, measured 4.2-5.6 μ in thickness; Pycnidia partially submerged in the substratum, dark brown to black, globose to subglobose or elliptical; Ostiolate, measured 169.0-264.5 μ in diameter; Pycniophores arranged in a palisade-like layer on the inner surface of pycnidial wall with rounded tips and measured 5.0-7.2 x 2.0-2.2 μ in size; Pycniospores ooze out from ostiole, elliptical to ovate, round, one celled, hyaline to light brown, and measured 3.2-5.4 x 2.2-3.0 μ in size; Sclerotia not observed.

***Rhizoctonia bataticola* (Taub.) Butl.**

Dark brown to olivaceous black growth of the fungus was observed that covered the whole surface of the seeds. Sometimes darkbrown to black sclerotia were also observed on infected seeds.

Colonies on PDA fast growing, compact, fluffy, abundant aerial mycelium of white in initial stage which later turn into dark grey to olivaceous brown containing dark brown to black sclerotia; Reverse of the colony dark brown; Mycelium septate, hyaline when young later changed into dark brown in colour, 1.5-6.8 μ in thickness, branched, branching at right angle with constriction at the point of branching alongwith a septum close to point of branching; Sclerotia abundant, olivaceous black, variable in shape and size, mostly spherical to

globose, 60.2-223.0 μ in diameter, either aggregated in grounds or formed singly; Chlamydospores present in chanins of 8-11, intercalary, spherical, oval to dome shaped; spherical 10.4-16.8 μ in diameter and dome shaped 10.4-20.0 x 6.8-14.2 μ; Sporodochia not observed.

***Rhizoctonia solani* Kuhn**

Characteristic mycelial growth of the fungus was observed on seed surface having light to dark brown mycelium. Dark brown sclerotia were also developed in the colonies.

Colonies on PDA fast growing, aerial mycelium light to dark brown, later on, produced abundant sclerotia; Reverse of the colony pale ochraceous buff to cinnemon; Mycelium septate, brown, branching at the right angles with a constriction just after the branching or the first septum placed a few microns beyond the point of origin, hyphal branches frequently anastomosses, main branch hyphae 3.8-6.0 μ thick and short side branch hyphae of 4.6-7.2 μ in thickness; Sclerotia submerged, clearly visible from the under side of the disk, blackish brown in colour, hard, spherical, frequently aggregated in groups, 0.5-2.0 mm in diameter; Chlamydospores hyaline, brownish in groups, variable in shape, cylindrical or barrel shaped, produced in chains, 16.0-23.0 x 10.0 - 14.5 (av. 20.8 x 12.0) μ; Sporodochia not observed.

Rhizopus arrhizus **Fischer**

Greyish brown to dark brown colonies with little developed stolons were seen on seeds; Under stereoscopic binocular microscope short and pale rhizoids were also observed.

Colonies on MEA fast growing, greenish brown but less exuberance as observed in *Rhizopus nigricans* and produced little developed stolens on nodes which covered the surface of the medium within 3-4 days of inoculation; short and pale rhizoids also developed at the nodes and carrying sporangia from one place to other, or rarely formed indeterminately; Mycelium aseptate, branched, 4.8-7.5 μ, thick, rhizoids often develop where sporangiophores arise; Sporangiophores often prostrate, rarely single, aseptate, sometimes branched, dark brown in colour, forming umbels or corymbs on their stolens and measured upto 0.5-1.9 mm in length and 14.5 μ in thickness and did not extent so far into the substrate; Sproangia spherical, dark brown to black, 126.0-230.8 (av. 171.5) μ in diameter. Columellae spherical, brown, smooth, flattened on the apophyses, 39.5-71.0 x 56.0-95.0 (av. 53.5x68.0) μ; Sporangiospores round or oval or presenting obtuse angles, greyish brown in colour, walls striated longitudinally and measured 4.0-6.5 x 4.0-5.2 (av. 5.5 x 4.5) μ in size; Zygospores not observed.

Rhizopus nigricans **Ehreno.**

Grey coloured colonies with well developed and hyaline stolens which

become brown towards the nodes, covered surface of the seeds. These stolens produced numerous sporangiopheres and rhizoids from the nodes which distinguishes from *Mucor*.

Colonies on MEA grew rapidly and sporulate freely by the production of stolens from the nodes of both rhizoids and sporoangiophores; Mycelium aerial, copious, loose which was spread out on the lid of the Petridish, if produced, more or less branched; Stolens hyaline, creeping, recurving to the substrate arachnoid hyphae which were strongly raised and distinct from the substrate and implemented at each node by means of rhizoids; Rhizoids short, brown, sometimes absent; Sporangiophores arise in groups of 3 to 5 but rarely single, golden brown in colour, 495.0-1172.5 (av. 658.0) μ long and 7.5-24.0 μ thick; Sporangia subspherical, 97.5-168.0 (av. 119.5) μ in diameter; Columellae subspherical, depressed, light brown, 32.0-67.5 (av. 53.5) x 35.5-69.0 (av. 58.0) μ, under dry conditions it collapse and the sporangia then resemble the heads of minute toad stools; Sporangiospores continuous, unequal, golden brown, globose or oval, angular, striate, 5.5-12.0 x 4.0-5.0 μ in size; Zygospores not observed.

***Trichothecium roseum* Link ex. Fries**

Colonies appeared as a pink crust on the seeds, superfecially, resembling the spore mass of *Fusarium*. Conidiophores having conidia in besipetal succession from the tips also observed under stereoscopic binocular microscope.

Colonies on MEA grew fast, smooth, with a rather powdery appearance, having numerous glistening conidia, young colony white which later becoming pinkish due to copious sporulation and finally pink; zonations indistinguished; Mycelium septate, hyaline, branched, made of creeping hyphae, 3.0-3.8 μ thick; Conidiophores arise singly from the mycelium, errect, long and slender, hyaline, septate, unbranched with little swelling at their tips, 212.6-358.0 x 3.0-3.8 μ in size; Conidia formed in basipetal succession at the swelling of the tips of the conidiophore, smooth, hayline, pinkish in mass, more or less ovoid, 2-celled, slightly constructed at the septum; the region of the conidia attached to the conidiophore was often blunt and ecentric, 13.0-23.0 (av. 18.0) x 6.0-10.5 (av. 7.5) μ.

The pathogenic behaviour of all the twenty-two fungal species isolated from Pigeonpea seeds was first tested in wide mouth glass tubes under laboratory conditions and then in pot experiments by soil inoculation and leaf inoculation methods as described under 'Materials and Methods' and the observations recorded are as follows.

The potential pathogenicity of different fungi was tested in wide mouth glass tubes on the seeds of Pusa 85. The nature and type of symptoms produced by each fungus are given in Table 6.

Out of twenty-two fungal species inoculated, *A. alternata, A. longissima, A. flavus, A. niger, B. cinerea, F. moniliforme, F. semitectum, P. Cajani, R. bataticola, R. solani* and *T. roseum* were found pathogenic to cause rotting of ungerminated and germinated seeds (Table 6). *A. alternata, A. flavus, A. niger, F. moniliforme* and *R. solani* were recorded as fast seed rotter whereas *A. longissima* and *P. cajani* were mild. The symptoms produced by each fungus are described below.

***Alternaria* species :**

A. alternata produced pre-emergence mortality due to rotting of ungerminated and germinated seeds. It produced brown rots on hypocotyl which ultimately lead to blight of seedlings. *A. longissima* caused only seed rot of ungerminated seeds.

Aspergillus species :

The seeds inoculated with *A. flavus* and *A. niger* produced rotting of seeds by covering while seed surfaces with pale green conidial mass in case of *A. flavus* and black coloured spore mass of *A. niger*. Besides, *A. flavus* was found to cause rotting of root and developing shoot leading to seedling mortality. Such type of seedling mortality was also noticed in Standard Blotter Method.

Botrytis cinerea :

This fungus caused rotting of ungerminated seeds only which ultimately reduced germination percentage.

***Fusarium* species :**

Both the *Fusarium* species caused extensive rotting of ungerminated and germinated seeds. *F. moniliforme* in addition, produced blighting of seedlings. The hypocotyl tissues of the affected seedlings became greyish brown in colour first and then rotted due to white to light pinkish growth on it. This fungus also produced rotting of roots and the affected seedlings get killed within 10 days *F. semitectum* caused rotting of germinated seeds by producing brown necrosis on the redicle. The affected seedlings ultimately get killed within 10 days of inoculation.

***Phyllosticta cajani* :**

This fungus caused seed rot. The rotted seeds were found completely covered with the fungal growth. *P. cajani* infested seeds produced elongated, brown necrotic lesions on the redicle soon after emergence followed by similar symptoms on emerging shoot. Few seedlings died due to heavy colonization of radicle and emerging shoot.

***Rhizoctonia* species :**

The mycelial growth of both, *R. bataticola* and *R. solani* grew fast and

soon cover the ungerminated and germinating seeds which in due course, colonized the developing radicle to produce dark brown rot symptoms leading to death of the germinating seeds. *R. bataticola* in addition, produced dark brown lesions on the hypocotyl of seedlings that lead to ultimately death of seedlings. The rotted seeds in case of *R. solani* were found covered with profuse mycelial growth. It also produced light brown lesions on the emerging shoot .

Trichothecium roseum :

It was found to cause rotting of both ungerminated and germinated seeds. The rotted seeds were fully covered with the white to pinkish growth of the fungus.

The remaining fungal species, such as *Cephalosporium sp., Chaetomium globosum, C. cladosporioides, C. dematium, C. lunata, D. tetramera, Mucor sp., F. oxalicum, Phoma sp.. R. arrhizus* and *R. nigricans* were not found pathogenic as these failed to produce any deterimental effect on seeds and seedlings. The uninoculated seeds in each case did not exhibit any symptom and produced healthy seedlings.

PATHOGENICITY TESTS

In most plant diseases, however, the amount of damage caused to plants is often much greater than would be expected from the mere removed of nutrients by the parasite. This additional damage results from substances secreted by the parasite or produced by the host in response to stimuli originating in the parasite. Tissues affected by such substances may show increased respiration, disintegration or collapse of cells wilting abscission, abnormal cell division and enlargement, and degeneration of specific components such chlorophyll. These conditions do not seem directly to improve the welfare of the parasite. It would appear therefore that the degree of pathogenicity exhibited by a parasite is not always proportional to the nutritional affiliation of the parasite and its host. Pathogenicity, then may be defined as the ability of the parasite to interfere with one or more of the essential functions of the plant, with parasitism frequently playing an important but not always the most important role (Agrios 1988).

Rajan *et. al.*, 2002, Pathogenicity experiments conducted showed the involvement of *Pythium sp.* (soft-rot), *Fusarium oxysporum* (dry-rot), *Ralslonia* (Pseudomonas) *Solnacearŭm* (wilt) and also noticed that *Pratylenchus coffeae* increased the severity of infection along with *F. oxysporum.*

The plants show signs of gradual wilting, as if affected by drought about five to six weeks after sowing. The leaves of affected plants turn yellow

prematurely, the foliage droops and within three or four days the plant wilts within six weeks plants of all ages may be affected. The disease first appears sporadically on a few plants, later spreading in concentric circles to nearby plants. In sever cases large patches of wilted plants can be found, frequently resulting in more than 50% dead plants in a field.

When diseased plants are pulled out and examined in the field, black lesions of varying size but mostly linear with regular margin may be seen on the stem and tap roots. The lesion and discoloration are deep seated and this becomes clear if the bark is peeled off, revealing the stem and root tissues in the affected portions as deeply black.

The unilateral wilt symptoms were also observed in various cases which clearly differentiate this pathological wilting phenomenon from physiological wilting. This may be due to the infection of roots and stems on one side only and the consequent confinement of the pathogen to that side only (due to restricted lateral movement of the pathogen). The common observation was that disease do not develop uniformly for example, if a branch has two shoots which are on opposite directions one may show wilting and drying of the leaves and other may have healthy green leaves.

The general health of the tree also deteriorates and some other important symptoms like poor growth of the young shoots and chlorosis of the older leaves

were also observed. In general it took 3-4 months for a healthy plants of the Arhar crops under investigation converting into a dried specimen with few mummified pods still attached to the dried branches along with source wilted (dried) leaves. The same species of Fusarium viz. *F. solani, F. oxysporum, F. acuminatum* and *F. equiseti* which commonly occur in soils of different fields arround Allahabad, Pratapgarh were also taken for pathological studies. These studies were carried out from the same stock cultures and hence the methods used for isolating subculturing etc., were similar to those described earlier pathogenicity tests were carried out by the following methods.

(1) The plants root both uninjured and injured (10 injuries by sterilized needle per root) were dipped in spore suspension (about 100 spores for lower field of compound microscope) of *Fusarium* species. The plants were transplanted in plastic pots. Control were simultaneously maintained.

(2) The plants were kept in culture tubes containing 20 days old culture filtrate of *Fusarium* spp. In case of control plants were kept in sterilized distilled water daily observation were made.

Reisolations were always made in order to confirm the infection with perticular *Fusarium* sp.

Ten plants per treatment were taken in each case. The results of both set of experiments are summarized in Table 7.

Results from the above pathogenicity test clearly show that all the four species of *Fusarium* sp. were capable of causing wilt of Arhar plants. The wilting was characterised by gradual withering, yellowing during of leaves. Later on it was observed that there was slightly higher percentage of infection injured root plants than those uninjured. Out of the four species in which *F. oxysporum* is more pathogenic than other three species as it caused a higher percentage of wilting in the seedlings.

Results from another set, where the seedlings were kept in culture filtrates of F. species, showed that seedlings wilted within seven days. All the seedlings kept in culture filtrated of *F. oxysporum* and *F. solani* wilted while in *F. acuminatum* the percentage was a little less controls remained healthy in both set of experiments thus from the above experiment it is clearly evident that *F. oxysporum* and *F. solani* causes more damage than other two species of *Fusarium* and from second set that some toxic substance also play a role in causing wilting of Arhar seedlings. The same results show in first set *F. oxysporum* was found more pathogenic than other three *Fusarium* sp. and another set *F. oxysporum* wilted while in other F. species was a little loss. Controls remained healthy in both set of experiments (Singh and Prasad, 1977).

Aqueous mycelial and spore suspensions of the organisms prepared from 7 to 10 days old cultures, were spored on injured as well as uninjured leaves and stems of their suspective host plants with the help of hand automizer. Humidity

was maintained by covering it with polythene bag with some sterilized water at the base. Suitable controls were maintained for each treatments.

Host Range

Pathogens differ with respect to the kinds of plants that they can attack, with respect to the organs and tissues that they can infect, and with respect to the age of the organ or tissue-of the plant on which they can grow. Some pathogens are restricted to a single species, other to one grows of plants while other have a wide range of hosts, including many taxonomic groups of higher plants. Some pathogens grow specially on roots, others on stems some mainly on the leaves or on fleshy fruit or vegetables. Some pathogens, for example, vascular parasites attack specially certain kinds of tissues such as vascular tissues. Others may produce different effects on different parts of the some plants. With regard to the age of plants, some pathogens attack seedlings on the young tender parts of plants, while attack only mature tissue.

Most obligate parasites are usually quite specific as to the kind of host they attack, possibly because they have evolved in parallel with their host and require certain nutrients that are produced or become available to the pathogen only in these hosts. Non obligate parasites usually attack many different plants and plant parts of varying age, possible because they depend for their attack on non specific toxins of enzymes that effect substances or processes found commonly among

plants. Some non-obligate parasites however produce disease on only one or a few plant species. In any case, the number of plant species currently known to be susceptible to a single pathogens is smaller than the actual number in nature since only a few species out of thousands have been studied for their susceptibility to each pathogen.

In order to as certain the range of parasites the wilt pathogens were inoculated one some common fruits because of the possibility that the infection on particular host may come from the plants growing invicinity.

A knowledge of the range of parasitism will be helpful in giving an-idea about the survival of the pathogenic fungi under adverse environmental conditions.

The results are recorded in table 8 and 9.

A persual of the table reveals that F. oxysporum could infect the seedling of *Cajanus cajan, Cicer arietinum, Pisum sativum, Vigna mungo, Vigna radiata* and *Lathyrus sativus, F. equiseti, F. acuminatum* could infect only *Cajanus cajan* and Chikpea but *F. solani* could infect *Cajanus cajan, Cicer arietinum, Pisum sativum* but failed to infect the seedlings of *Vigna mungo, V. radiata, Lathyrus sativus* by *F. equiseti* and *F. acuminatum* but *F. solani* could not infect only *Vigna radiata* and *Lathyrus sativus.*

Thus *F. oxysporum* and *F. solani* were found to have a wide host range.

Detection of Fusaric Acid

Fusaric acid was originally isolated from *Fusarium heterosporum* by Yabuta *et al.*, (1934) and shown to be butyl picalinic acid. It is non specific vivotoxin. Its production in vitro by a number of fungi, all belonging to family Hypocreacae has been demonstrated by (Gaumann 1957), *Fusarium oxysporum* (*F. lycopersici, F. vasinfectum F. niveum, F. batatae, F. nicotianae.*) (Gaumann *et al.*, 1952) *F. solani, F. moniliforme, F. majua* (Lakshminarayan and Subramanian, 1955) and *Nectria cinnarabarina* (Nishimura, 1957).

Much work has been by a number of workers including Chandramohan and Mahadevan (1968), Kuo and Scheffer (1964) and Sandhu (1960) about the importance of Fusaric acid related to wilt symptoms. Fusaric acid has been shown inhibit the electron transport in mitochondria.

Fusarium species were grown for 15 days in 100 ml. of Czapek's liquid medium in 500 ml. Erlermeyer flasks. The culture filtrates were collected and centrifuged at 2200 rpm for 20 minutes. The clear supernant was taken and the pH was adjusted to 4.0 by adding 2 NHCl 100 ml. of the filtrate was mixed with equal volumes of ethyl acetate at least for 4 times in a separating funnel allowing 15 minutes for each extraction. All ethyl acetate extracts were mixed and evaporated to dryness. 1-2 ml. of ethanol was added to dissolve the residue. Drops of known volume (0.005 ml.) of filtrates as well as an index solution of Fusaric acid were

kept on Whatman's filter paper No. 1 spotted chromatogram were sum ascendingly for 10 to 12 hours in butanol, formic acid and water (75 : 15 : 10). Chromatograms were dried and sprayed by bronophenol blue (0.04% in 90% ethyl alchol) Fusaric acid gives a yellow colour.

Fusaric acid was detected in all the present species of *Fusarium.* The amount produced by them, however, varied on the basis of visual comparison of the chromatograms, based on the fact that the size and colour intensity of the spots, the four species of *Fusarium* are graded as follows :

F. solani .... +++++++++

F. oxysporum .... ++++++++++

F. acuminatum ... ++++++

F. equiseti ... +++

Table : 3

Percent incidence of fungal species associated with different categories and grades of pigeonpea seeds of Var. UPAS 120 (Number of seeds tested : 200)

Fungal species	Categories					
	Asparently healthy looking seeds		Discoloured seeds		Shrivelled seeds	
	Under sized	Normal	Under sized	Normal	Under sized	Normal
Alternaria alternata	8	6	13	11	22	20
Aspergillus flavus	6	3	6	4	11	9
A. niger	4	2	6	5	10	6
Botrytis cinerea	2	-*	4	3	6	5
Cladosporium cladosporioides	-	-	3	-	8	5
Colletotrichum dematium	4	2	4	3	6	5
Curvularia lunata	2	-	3	-	4	6
Drechslera tetramera	-	-	4	3	10	6
Fusarium moniliforme	7	4	6	4	14	12
F. semitectum	8	4	7	5	11	10
Mucor sp.	-	-	-	-	4	2
Penicillium oxalicum	-	-	2	1	3	3
Phoma sp.	-	-	4	2	6	3
Phyllosticta cajani	5	2	7	6	10	9
Rhizoctonia bataticola	4	3	10	7	10	8
R. solani	8	5	8	6	10	8
Rhizopus arrhizus	1	1	4	3	5	4
R. nigricans	-	-	3	2	4	2
Trichothecium roseum	-	-	3	2	6	2
Total number of fungal species	12	10	18	16	19	19
Total number of fungal colonies	59	32	97	67	160	125

*- Denotes Absence

Table : 4

Percent recovery of fungal species from the internal tissue of seed

(Number of seeds tested : (400)

Fungi	Percent seed infection	
	UPAS 120	Pant A 8508
Alternaria alternata	12	7
A. longissima	-*	2
Aspergillus flavus	4	-
Cladosporium cladosporioides	1	-
Collectotrichum dematium	3	-
Curvularia lunata	3	-
Fusarium moniliforme	6	-
F. semitectum	4	2
Phyllosticta cajani	2	-
Rhizoctonia bataticola	5	-
R. solani	4	-
Trichothecium roseum	2	-

*- Denotes Absence

Table : 5

Percent seed infection of different fungal species in seed components of pigeonpea (Number of seeds tested : 100)

Fungi	No. of infected seeds		Infection in seed components of pigeonpea					
	UPAS 120	Pant A 8508	Seed coat		Cotyledons		Embryonal axis	
			UPAS 120	Pant A 8508	UPAS 120	Pant A 8508	UPAS 120	Pant A 8508
Alternaria alternata	16	12	16	12	-*	-	-	-
A. longissima	-	5	-	5	-	-	-	-
Aspergillus flavus	8	-	8	-	3	-	-	-
Cladosporium cladosporioides	5	-	5	-	-	-	-	-
Colletotrichum dematium	4	-	4	-	2	-	-	-
Curvularia lunata	5	-	5	-	-	-	-	-
Fusarium moniliforme	13	-	13	-	6	-	4	-
F. semitectum	9	6	7	5	4	3	-	-
Phyllosticta cajani	7	-	7	-	7	-	2	-
Rhizoctonia bataticola	8	-	8	-	5	-	3	-
R. solani	6	-	6	-	4	-	2	-
Trichothecium roseum	5	-	4	-	-	-	-	-

*- Denotes Absence

Table : 6

Effect of different fungal species on the health of seeds and seedlings under laboratory conditions (Number of seeds inoculated : 10)

S.No.	Fungal species	No. of seeds germinated	No. of seeds rotted	No. of seedlings diseased	Pathogenic effect
1.	*Alternaria alternata*	8	3	3	Seed rot, seedling blight
2.	*A. longissima*	8	2	–	Seed rot
3.	*Aspergillus flavus*	6	4	5	Seed rot, root rot, seedling mortality
4.	*A. niger*	8	2	–	Seed rot
5.	*Botrytis cinerea*	8	2	–	Seed rot
6.	*Cephalosporium sp.*	10	–	–	No effect
7.	*Chaetomium globosum*	10	–	–	No effect
8.	*Cladosporium cladosporioides*	10	–	–	No effect
9.	*Colletotrichum dematium*	10	–	–	No effect
10.	*Curvularia lunata*	10	–	–	No effect
11.	*Drechslera tetramera*	9	1	–	No effect
12.	*Fusarium moniliforme*	6	4	–	Seed rot, Seedling mortality, necrosis of radicle
13.	*F. semitectum*	8	1	1	Seed rot, root rot, seedling mortality
14.	*Mucor sp.*	10	–	–	No effect
15.	*Penicillium oxalicum*	10	–	–	No effect
16.	*Phoma sp.*	10	–	–	No effect
17.	*Phyllosticta cajani*	8	1	3	Seed rot, brown spots on hypocotyl
18.	*Rhizoctonia bataticola*	9	1	5	Seed rot, seedling mortality, lesions on hypocotyl
19.	*R. solani*	6	3	2	Seed rot, root rot, seedling blight
20	*Rhizopus arrhizus*	9	–	–	No effect
21.	*R. nigricans*	9	–	–	No effect
22.	*Trichothecium roseum*	8	1	–	Seed rot
23.	*Control*	10	–	–	No effect

Table : 7

Showing Pathogenicity Results

Pathogen	Disease	Symptoms
Fusarium oxysporum	Wilt and seedling rot	The leaves of affected plants turn yellow pre-maturely, the foliage droops and within three or four days the plant dry up. The first symptoms of the disease is yellowing of the leaves. Within a day or two such leaves drop and in the course of next two or three days they may drop off, when the stem is examined closely, dark lesions may be seen on the bark at the ground level.
F. solani	Wilt and root-rot	Plant show stunting dropping and chlorosis of leaves. Stem also shows shrinkage in lower portion roots are poorly developed
F. equiseti	Root-rot	Root start gradual, roots poorly develop and finer root lets get destroyed.
F. acuminatum	Wilt and Root-rot	Lack of root hairs and other secondary roots, yellowing and shrivelling of leaves and finally wilting.

Table : 8

Results of cross inoculation with different *Fusarium* sp. on various pulses seedling

S.No.	Pulses (seedlings)	*F. oxysporum*	*F. equiseti*	*F. solani*	*F. acuminatum*
1.	*Cajanus cajan*	+	+	+	+
2.	*Cicer arietinum*	+	+	+	+
3.	*Pisum sativum*	+	-	+	-
4.	*Vigna mungo*	+	-	-	-
5.	*Vigna radiata*	+	-	-	-
6.	*Lathyrus sativus*	+	-	-	-

Table : 9

Pathogenicity test and percentage wilting of Arhar (*Cajanus cajan*) seedlings

S.No.	Organism	Percentage of wilting		
		After 1 Week		After 4 days in culture filtrate
		Uninjured	Injured	
1.	*Fusarium solani*	40	50	70
2.	*F. oxysporum*	55	65	100
3.	*F. acuminatum*	50	55	60
4.	*F. equiseti*	20	25	55
	Control	Nil	Nil	Nil

Chapter 4
Physiological Studies

Wilt of Arhar (*Cajanus cajan*)
Fusarium solani

PHYSIOLOGICAL STUDIES

Fungi are dependent on the medium or the substrate for all the elements and compounds which they require or utilize, except molecular oxygen and possibly a little carbondioxide which are obtained from the atmosphere. Nutritional requirements of various fungi differ and there is no one medium or substrate which can be universally suited to all the fungi. The genetic constitution of the fungus determines what it can do, but the expression of its potentialities is dependent upon the composition of the medium on which it grows and the environment to which it is exposed. The choice of the media and environmental condition depend on the purpose of the investigation. Micheli (1679-1737) was the first botanist who cultured fungi on pieces of water-melones. The use of such substrate for the artificial culture of fungi continued till Pasture (1860) used what might be considered a proximation of a chemically defined medium during his studies on alcoholic fermentation. Credit goes to Roulin (1869), with *Aspergillus niger* a student of Pasteur, who first devised a synthetic medium for the nutritional studies of some common fungi. Lilly and Barnett (1951) found that all media were not equally suited for a particular fungus.

Bilgrami (1956) working with *Phyllosticta cycadina, P. artocarpina* and *Pestalotia mangiferae*, observed that the best growth of *Phyllosticta cycadina* was on Richard's medium followed by Oatmeal medium, whereas *P. artocarpina* and *Pestalotia mangiferae* showed best results on Richard's medium followed by

Potato Dextrose-Agar medium. The sporulation of all of them was best on Asthana and Hawker's medium 'A', while it was good on Potato Dextrose and Oatmeal media and was poor on Richard's medium.

The four *Fusarium* species under investigations were, therefore, grown on a number of media and their mycelial growth as well as sporulation was studied so that a suitable basal medium be selected for the maintenance and physiological studies.

The following media were under investigations.

(1) **Peptone Dextrose - Rose Bengal Agar (Martin, 1950)**

Agar	20.0 gm.
$K H_2PO_4$	1.0 gm.
$MgSO_4 . 7H_2O$	0.5 gm.
Peptone	5.0 gm.
Dextrose	10.0 gm.
Rose Bengal (1%)	3.3 ml.
Water (distilled)	1000.0 ml.
Streptomycin	30.0 mg.

(2) **Czapek-Dox-Agar (Raper and Thom, 1949)**

Agar	15.0 gm.
$NaNO_3$	2.0 gm.
K_2HPO_4	1.0 gm.
$MgSO_4 . 7H_2O$	0.5 gm.
$FeSO_4 . 7H_2O$	10.0 gm.

KCl	0.5 gm.
Sucrose	30.0 gm
Water (distilled)	1000.0 ml.

(3) **Oatmeal Agar (Johanson and Curl, 1972)**

Agar	17.0 gm.
Oatmeal	15.0 gm.
Yeast extract	1.0 gm.
Water (distilled)	1000 ml.

(4) **PCNB Agar, Modified (Papavizas, 1967)**

Agar	20.0 gm.
Peptone	5.0 gm.
KH_2PO_4	1.0 gm.
$MgSO_4 . 7H_2O$	0.5 gm.
Water (distilled)	1000 ml.
Oxgall	1.0 gm.
PCNB	0.5 gm.
Chlorotetracyclene HCl	50.0 mg.
Streptomycin Sulphate	100.0 mg.

(5) **Potato Dextrose Agar (Riker and Riker, 1936)**

Agar	17.0 gm.
Potato (Peeled and Sliced)	200 gm.
Dextrose	20 gm.
Water (distilled)	1000 ml.
pH	6.0-6.5

200 gm. of clean potatoes were peeled and cut into small pieces and boiled for half hour. 20 gm. of Dextrose was then added and the total volume of the medium was adjusted 1000 ml.

(6) Asthana and Hawker's medium 'A'

Glucose	5.0 gm.
KNO_3	3.5 gm.
KH_2PO_4	1.75 gm.
$MgSO_4 . 7H_2O$	0.75 gm.
Distilled water	1000 ml.

(7) Modified Asthana and Hawker's medium 'A'

Glucose	10.0 gm.
KNO_3	3.5 gm.
KH_2PO_4	1.75 gm.
$MgSO_4 . 7H_2O$	0.75 gm.
Distilled water	1000 ml.

(8) Richard's medium

KNO_3	10.0 gm.
KH_2PO_4	5.0 gm.
$MgSO_4 . 7H_2O$	2.5 gm.
$FeCl_3$	0.02 gm.
Sucrose	30.0 gm.
Distilled water	1000 ml.

(9) Glucose Asparagine medium

Glucose	2.0 gm.

Asparagine	2.0 gm.
K_3NO_4	1.25 gm.
$MgSO_4 . 7H_2O$	0.75 gm.
Distilled water	1000 ml.

Effect of different media

Fungi are non-vascular plants which lack chlorophyll. They are incapable of an independent existence as they are unable to synthesize their own food. Physiological studies of such organisms may be helpful to revealing the pathway of utilization of various substances and this may help in controlling the disease.

The average dry weight, degree of sporulation, chlamydospore formation and change in the pH by *Fusarium acuminatum, F. oxysporum, F. solani* and *F. equiseti* are recorded. Results from the tables (10, 11, 12 and 13) show Richard's medium supported best growth of the present species of *Fusarium*. It was followed by Czapek's medium in case of *F. acuminatum* and *F. solani* whereas PCNB Agar modified was second best in case of *F. oxysporum* and *F. equiseti.* Asthana and Hawker's medium 'A' supported poor growth of present fungi amongst the nine media tried.

Sporulation of the present organisms was excellent on modified Asthana and Hawker's medium 'A', Potato Dextrose-Agar and Richard's medium. It was poor on glucose asparagine, Oat meal and Pepton-Dextrose Rose Bengal Agar medium. Sporulation of the present species of *Fusarium* ranged from fair to good

on other media, chlamydospores were also observed in a few cases and were produced in fair degree on oat meal medium in each case. On Peptone Dextrose Rose Bengal medium only microspores of the present fungi were observed.

The final pH of different media increased. However a decrease in pH was recorded in case of Czepek's Dox-Agar medium and Oatmeal media in every case.

Among of the nine media used, modified Asthana and Hawker's medium 'A' was selected as basal medium for the present four species of *Fusarium* as it provided fair growth and excellent sporulation.

EFFECT OF TEMPERATURES

Temperature is one of the most important physical factors which influences metabolic activity of fungi. The fungi grow within a certain range of temperature which varies from organism to organism. Togashi (1949) has given a list of temperatures requirement for a large number of fungi which play a significant role in covering various metabolic activities of the micro-organisms. According to Togashi (1949) the optimum temperature for fungal growth lies in between 20°C and 30°C. Usually fungi do not grow below 0°C or above 40°C but exceptions are not infrequent. Broad foot and Cormack (1941), Remberg (1940) and Dahl (1934) reported that some fungi are able to grow slowly at 0°C or slightly less and even plants under snow may be infected by parasitic "snow mould" *Typhula incarnata*, *Fusarium nivole* and an unidentified basidiomycetes respectively. Pehrson (1948) observed that *Phacidium infestan* which causes a disease of pines, is able to grow in culture at -30°C. Bidault (1921) as well as Brooks and Hansford (1923) reported that *Cladosporium harbanum* could grow slowly at 6°C and even slightly at -1°C on the other hand, Hawker (1950) observed the growth of *Sordaria* sp. at 40°C-42°C and La Touche (1948) reported a *Chaetomium* like fungus to grow even at 62°C.

The range of temperature over which sporulation occurs is, usually narrower than that permitting mycelial growth. Barnett (1921) observed the mycelial growth of *Phoma apicola* between 5°C to 28°C while pycnidial development look place between 13°C to 26°C. Ames (1915) reported that the range for growth of *Monilia fructigena* and *Cephalothecium roseum* were 4°C-30°C and 9°C-35°C respectively while the corresponding ones for the production of conidia were 9°C-30°C and 14°C-30°C.

Tisdale (1917) found that minimum temperature for growth of *Fusarium lini* was 10°C and the optimum was 25°C-28°C. Studies made by Edson and Shapolov (1920) showed that the minimum, optimum and maximum temperatures for *F. coeruleum* and *F. trichothicioides* were 5°C, 25°C and 35°C respectively. Moore (1924) working with *F. coeruleum* failed to get any growth of the fungus at 5°C. The optimum being 20°C to 25°C Massey (1926) observed that *F. oxysporum* on Gladioli could grow over a range of 5°C to 35°C with optimum temperature at 27.5°C. Agarwal (1955) reported that *F. coeruleum* could not sporulate at 8°C but the sporulation was best at 20°C-24°C and it decreased at higher temperatures.

From the above mentioned facts it would be evident that before starting any physiological experiment, it is indispensable to have a thorough knowledge the temperature requirements of the organism concerned. Moreover, this would also give an idea about the environmental condition that would be most suitable for survival and propagation of a pathogen in nature. Therefore it was considered

necessary to determine the cardinal temperatures and especially the optimum temperature for growth and sporulation of the present isolates. The following temperatures was taken : 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 40°C. The results are summarized in tables 14, 15, 16 and 17.

Results from the tables (14, 15, 16 and 17) clearly show that *F. acuminatum, F. oxysporum, F. solani* and *F. equiseti* could grow between a range of 10°C to 35°C. Poor growth of the four species was recorded at 10°C, while a high temperature 35°C supported moderate growth. Good growth of all four *Fusarium* species was observed between 20°C to 30°C. Both, growth and sporulation of the present fungi were optimum at 25°C.

Sporulation as well as chlamydospore formation of the present organisms were prominently influenced by the temperature variations. Sporulation varied from poor to excellent in all cases except in *F. oxysporum* which failed to sporulate at 10°C. *Chlamydospore* formation was better in all the four species when the temperature was unfavourable 10°C to 35°C.

EFFECT OF pH VARIATION

Hydrogen - ion concentration of the medium has a profound influence on overall metabolic activities in fungi. It affects permeability of protoplasmic membrane, uptake of minerals, entry of essential vitamins and organic acids into the cells, activities of enzyme systems, synthesis and stability of proteins and other similar life processes. The pH of the substrate also influences the processed like production of pigments, vitamins, antibiotics and other metabolics.

The growth and reproduction of fungi are profoundly influenced by the hydrogen-ion concentration of the substrate upon which they grow. Highly acidic or alkaline media inhibit the growth of different fungi. Few fungi are, however reported to grow at pH 1 or as high as pH 12, but there are some exceptions.

Lilly and Barnett (1951) mentioned that most suitable range for the growth of many fungi varied between pH 5 to pH 6. Yu (1954) reported that *Ascobolus magnificus* was able to grow over the pH range of 3-12. Hawker (1950) observed that most of the fungi grew best at neutral reaction i.e., pH 7 or slightly on acid side. Exceptionally low pH value has some times been obtained as optimum for some fungi, particularly *Aspergillus* and certain wood destroying fungi. Meacham (1918) reported that the optimum pH for the growth of *Lensites saepiaria, Fomes roseus, Merulius lagrymans and Coniophora cerebella* was 3. On the other hand, some fungi like *Fusarium bullatum* (Johnson, 1923), *F. coeruleum* (Agarwal,

1955) and *Glaeosporium limettieolum* (Agarwal, 1955) have been reported to grow even at pH 11.

Asthana and Hawker (1936) working with *Melanospora destruens* observed that the growth of this fungus was restricted to a relatively narrow range of pH 4.8 to 7.6. Agarwal (1955) found that pH range for two strains of *Fusarium coeruleum* was between 3.4 to 11.0 and pH 6.4 was optimum for growth and sporulation. Sherwood (1923) found that *Fusarium lycopersici* could grow within a pH range of 2.2 to 8.4. Srinivaspai (1953), reported that *Fusarium vasinfectum* and *F. moniliforme* could grow at considerably lower pH of 2.1

In view of all these findings it was considered desirable to study the effect of initial pH of the medium on growth and sporulation of the fungi included in the present investigation. The pH of modified Asthana and Hawker's medium 'A' was varied from 8.0 to 11.0 and following different pH were employed.

2.0, 2.5, 3.0, 3,5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 8.0, 9.0, 10.5, 11.0

The final pH after the growth was also recorded and the results obtained have been summarised in Tables 18, 19, 20 and 21.

A persual of the above tables indicates that all the four *Fusarium* sp. could grow and sporulate over a wide range. The organisms failed to grow at lower pH viz., 2.0. The maximum dry weight of *F. acuminatum, F. oxysporum, F. solani* and *F. equiseti* was attained at pH 5.5 significant good growth of all the four *Fusarium*

sp. was observed on the acidic side. Sporulation of the *Fusarium* sp. was excellent from pH 5.0 to 6.0 while it was absent at pH 2.5. But sporulation of *F. equiseti* was excellent from pH 5.0-6.5.

Chlamydospores of *F. acuminatum* were observed at pH values between 4.0 to 11.0. In *F. oxysporum* and *F. solani* they appeared between pH 2.5 to 11.0, but in *F. equiseti Chlamydospore* appeared between pH 4-11. No chlamydospores were observed at pH 5.5 in all the four *Fusarium* sp., change was observed in the final pH of the media. It was found that in all those cases where the original pH was low the final pH increased but in those cases where it was high the pH decreased.

Optimum growth and excellent sporulation of all the four organisms were observed at pH 5.5. Tandon (1961) and his collaborators obtained similar results for a number of fungi studied by them. Yogeshwari (1948) reported that optimum pH for *Fusarium vasinfectum, F. udum* and *F. moniliforme* was 8. Joffe and Palti (1972) observed that in culture, isolates of *F. solani* and *F. javanicum* grew better at pH 7 than pH 4.2. Cochrane and Cochrane (1971) observed that proportion of chlamydospore fell lineraly in case of *F. solani* between pH 4.0 to 6.5.

Tables (18, 19, 20 and 21) show lucidity that the pH of the medium of all the four *Fusarium* sp. tended to drift towards neutral point or slightly alkaline side at the end of incubation period.

In highly acidic media this drift was towards less acidic side and in highly alkaline media this was towards lesser alkalinity. Hawker (1950) reported that the growth of *Fusarium fructigenum* on Richard's solution, which is normally acidic (pH 4.6), gradually increased the pH until the reaction was strongly alkaline. Ramkrishnan (1942) working with *Collatotrichum falcatum* observed that whatever be the initial pH value of the nutrient medium, the final values were always around the neutrality. Similar results were observed by Muller (1962) for *Gloeosporium musarum* and *G. fructigenum,* Agnihotri (1963) for *Colletotrichum capsici.* The rise in pH of the culture medium has been attributed to the metabolic activities during growth resulting in absorption of anion or production of ammonia from nitrogenous compounds. Lowering of pH in case of media with higher initial pH was possibly due to absorption of carbon dioxide produced by the fungus in the process of respiration.

Good growth of the four fungi under investigation was obtained on the acidic side or near about neutral. Similar results have been reported by Bilgrami (1956), Tandon and Bhargava (1960), Chaturvedi (1961), Bhargava (1962), Jamaluddin (1973) and Khanna (1974), who have also reported that the growth of these organisms was more favourable on the acidic media.

On the basis of the above investigation the pH of the media for all the further work with the four *Fusarium* sp. was adjusted at 5.5.

EFFECT OF SOME VITAMINS

Vitamins are organic substances and are needed in a minute quantity for the growth of an organism. The role of vitamins in promoting growth of various organisms was known from the ninetieth century and they have been referred in the literature as growth promoting substances, accessory growth factors, auximones, auxithals, nutrilites etc. The name 'Vitamins' was given to these substances by Funk, (1914) and in recent years this term has become most conventional for designating these growth promoting substances. According to Fries (1965) vitamins do not serve merely as a source of energy but they are necessary for the growth of different organism in a minute quantity. When used in case of fungi they function as part of coenzyme.

Like other organisms, fungi also require minute amount of vitamins for their growth. Lilly and Barnett (1951) mentioned that the "Characteristic feature of a growth factor (vitamin) include the following (1) its organic nature; (2) its activity in minute amounts; (3) its catalytic action, and (4) the specificity of its action".

A vitamin deficient fungus can be completely incapable of synthesizing a certain growth factor, or the biosynthetic capacity may be reduced which may be gained by an external supply of a favourable vitamin in the substrate.

Fungi which are unable to synthesize the vitamins are called vitamin-deficient. Schopfer (1943) called them hetrotrophic fungi with respect to vitamins.

A number of fungi are known to be vitamin-deficient but the needs of the various species differ and may exhibit partial or total as well as single or multiple deficiency. On the other hand fungi may or may not be dependent partially or totally on an exogenous supply of vitamins. Many of them including species of *Fusarium, Aspergillus* and *Penicillium* are able to grow on vitamin free substance and the addition of the vitamin may not significantly affect their growth. Such forms which do not need external supply of vitamins, are known as self-sufficient. Schopfer (1943) called such fungi as 'autotrophic' with respect to vitamins.

A number of workers including Hawker (1938), Robbins and Kavanagh (1942), Leonian and Lilly (1942), Lilly and Barnett (1949), Mathur *et al.* (1950), Backman et al. (1953), Sadasivan and Subramanian (1954), Tandon and Bilgrami (1957 C), Saksena (1958), Misra and Mahmood (1961), Hackbarth and Collins (1961), Collins and Hackbarth (1962), Ward (1962), Vail (1962), Bhargava and Tandon (1963), Bilgrami (1963), Hine and Aragaki (1963), Goldstrohm (1963), Kaisar (1964), Prasad (1966), Joshi and Haware (1966), Dayal and Joshi (1969), Gupta *et al.* (1970), Hedge and Ranganathllah (1971), Jamaluddin (1973), Khanna (1974) as well as Shiv Kumar (1975) have shown the importance of vitamins in the nutrition of fungi.

Generally, an exogenous supply of vitamins accelerate the growth of fungi, though some of the organisms are reported to attain the same mycelial growth even on a vitamin free medium. Usually the addition of vitamins especially at high

concentration exerts a harmful or inhibitory effect on the growth of the fungi.

In the present investigation an attempt has been made to study the effect of some vitamins on the growth and sporulation of four *Fusarium* species. Three sets of media were employed for the study. First set comprised of media without any vitamins, second set contained all the vitamins included in the study, while the third set contained only one vitamin at a time.

Vitamins were added in the basal medium at the following concentrations :-

Ascorbic acid	50.0 µg
Biotin	10.0 µg
Folic acid	20.0 µg
Nicotinic acid	50.0 µg
Riboflavin	50.0 µg
Thiamine hydrochloride	100.0 µg

The other details of the procedure have been given in the chapter dealing with "Materials and Methods". The results are recorded in the Table 22, 23, 24 and 25.

The present study revealed that all the four *Fusarium* species attained moderate growth on vitamin free media. Addition of a mixture of vitamins, however, increased the dry weight of all four species of *Fusarium* increased the dry weight of *Fusarium equiseti*, while it showed no effect on *F. oxysporum* and

F. solani. Results were also obtained by Lilly and Barnett (1951) for *Sphaeropsis malorum, Phoma betae,* Eveleigh (1961) for *P. violaceae*.

Individual effect of Vitamins on the four species of *Fusarium*

Ascorbic acid in general, none of the *Fusarium* species needed this vitamin. The addition of ascorbic acid exerted a inhibitory effect on the growth of *F. acuminatum* and *F. oxysporum* as it could induced poor growth for these organisms. Tandon (1950) working with *Pestalotie malorum* and *F. psidii* found that ascorbic acid inhibited the growth of above fungi. This vitamin, however, had no adverse effect on the growth of *F. solani*. The final pH of the medium used by *F. acuminatum* and *F. solani* drifted towards alkalinity but remain neutral in case of *F. oxysporum* and *F. equiseti.*

Biotin

Lilly and Barnett (1947), Backman *et al.* (1953), Sadasivan and Subramanian (1954), Hackbarth and Collins (1961), Tandon (1961), Ghosh (1964) and Kakkar (1964) found that biotin was important for the growth of the organisms studied by them. In the present investigation biotin accelerated the growth of *F. acuminatum.* Similar results were also obtained by Mathur *et al.* (1964) for *F. oxysporum* and *F. cuminis*. However, this vitamin had no effect on *F. oxysporum* and *F. solani* and *F. equiseti.*

The final pH of the medium moved towards alkalinity in all cases.

Folic Acid

The dry weight results show that this vitamin had no marked effect on the growth of present organisms as their dry weight were statistically similar to that produced on medium devoid of vitamins. The pH of the medium in all cases drifted towards alkalinity.

Nicotinic Acid

This vitamins showed the inhibitory effect on the growth of *F. acuminatum* and *F. oxysporum*. Tandon (1950) also recorded the inhibitory effect of nicotinic acid on the growth of *Pestalotia malorum* and *P. psidii*. However, it enhanced the growth of *F. solani* and *F. equiseti*. The final pH of the medium used by *F. acuminatum, F. oxysporum, F. solani* and *F. equiseti* was 7.0, 6.8, 7.5 and 7.5 respectively.

Riboflavin (Vitamin B_2)

F. acuminatum and *F. oxysporum* obtained good growth on riboflavin, whereas, it had no marked effect on the growth of *F. solani* and *F. equiseti*. The former two species researched *F. oxysporum, F. acuminis* (Mathur *et al.*, 1964). The final pH of the medium drifted towards alkalinity in all cases.

Thiamine (Vitamin B_1)

F. acuminatum and *F. oxysporum* attained good growth on this vitamin,

whereas, it showed inhibitory effect on the growth of *F. solani* and *F. equiseti*. The former two species resembled with organisms studied by Leonian and Lilly (1938), Robbins and Kavanagh (1938), Robbins and Ma (1942), Neocker and Read (1943), Lindeberg (1946), Lilly and Barnett (1947), Cantino (1948), Fries (1948), George (1951), Mathew (1952, 1953), Albritton (1953), Sadasivan and Subramanian (1954), Gough and Lilly (1956), Surayanarayan (1958), Goldstrohm (1963) as well as Prasad (1966). However, *F. solani* resembled with the organisms studied by Schopfer (1935), Lilly and Barnett (1949) Elliot (1949), Mathur *et al.* (1950) and Esposito *et al.* (1962) who have reported inhibitory effect of thiamine on the various fungi studied by them.

The final pH of the medium used by *F. acuminatum, F. solani and F. equiseti* drifted towards alkalinity, whereas it remained in acidic range in case of *F. oxysporum.*

Though external supply of individual vitamins had affected growth of the present species of *Fusarium* yet no marked difference in sporulation was observed. Sporulation of all the four species of *Fusarium* in general varied from excellent to good in media added with an individual or mixture of vitamins or devoid of vitamins.

It can be concluded from the present study that all the four *Fusarium* species were capable of synthesizing the essential vitamins needed for their growth

as all of them grow moderately on vitamin free medium. Out of four species of *Fusarium, F. oxysporum, F. solani* and *F. equiseti* attained similar growth on a mixture of vitamins and control (no vitamin added). However, the growth of *F. acuminatum* was enhanced in the vitamin mixture. This clearly shows that the above fungus could synthesize the vitamins necessary for its growth but addition of vitamins accelerated the growth. The results from the above study show that individual exogenous supply of a vitamin either did not influence the growth of the present organisms, or has accelerated it or exerted an inhibitory effect.

Table : 10

Showing the average dry weight, sporulation, change in pH and chlamydospore formation of *F. acuminatum* in different media

Different Media	Dry wt. in mg.	Sporulation	Chlamydospore formation	pH	
				Initial	Final
Asthana Hawker's medium 'A'	62.5	Excellent	Poor	5.2	6.5
Modified Asthana and Hawker's medium 'A'	74.8	Excellent	Poor	5.2	7.5
Czapek-Dox Agar medium	143.1	Good	--	7.0	6.4
Glucose Asparagine medium	97.0	Poor	Poor	5.1	5.5
Oat meal medium	128.9	Poor	Fair	6.2	5.5
Peptone Dextrose Rose Bengal Agar medium	65.3	Poor	Poor	5.5	6.1
PCNB Agar modified	99.7	Good	--	5.4	7.4
Potato Dextrose Agar medium	112.1	Excellent	--	6.1	6.1
Richard's medium	253.2	Excellent	--	6.4	6.2

Table : 11

Showing the average dry weight, sporulation change in pH and Chlamydospore formation of *F. oxysporum* on different media

Different media	Dry wt. in mg.	Sporulation	Chlamydospore formation	pH	
				Initial	Final
Asthana & Hawker's medium 'A'	43.7	Good	--	5.1	6.2
Modified Asthana & Hawker's medium 'A'	56.6	Excellent	Poor	5.0	6.8
Czapek Dox Agar medium	132.6	Good	--	7.5	6.8
Glucose Asparagine medium	57.0	Poor	Poor	5.0	5.2
Oat meal	124.9	Poor	Fair	6.5	5.5
Peptone Dextrose	57.7	Poor	Poor	5.5	6.0
Rose Bengal Agar medium					
PCNB Agar modified	156.0	Fair	--	5.5	8.8
Potato Dextrose Agar medium	150.0	Excellent	--	6.2	6.5
Richard's medium	248.3	Excellent	--	5.0	6.5

Table : 12

Showing the average dry weight, sporulation change in pH and Chlamydospore formation of *F. solani* on different media

Different media	Dry wt. in mg.	Sporulation	Chlamydospore formation	pH	
				Initial	Final
Asthana & Hawker's medium 'A'	47.1	Good	Poor	5.1	7.0
Modified Asthana & Hawker's medium 'A'	60.5	Excellent	Poor	5.0	7.9
Czapek Dox Agar medium	160.1	Excellent	7.5	7.2	6.8
Glucose Asparagine medium	85.2	Poor	Poor	5.5	6.2
Oatmeal	119.8	Poor	Fair	6.5	6.0
Peptone Dextrose Rose Bengal Agar medium	65.2	Poor	Poor	6.0	6.5
PCNB Agar modified	130.0	Fair	--	6.0	8.5
Potato Dextrose Agar medium	142.3	Excellent	--	6.5	6.5
Richard's medium	247.2	Excellent	--	5.0	6.5

Table : 13

Showing the average dry weight, sporulation change in pH and Chlamydospore formation of *F. equiseti* on different media

Different media	Dry wt. in mg.	Sporulation	Chlamydospore formation	pH	
				Initial	Final
Asthana & Hawker's medium 'A'	40.1	Good	--	5.5	6.5
Modified Asthana & Hawker's medium 'A'	53.6	Excellent	Poor	5.1	6.7
Czapek Dox Agar medium	130.0	Poor	--	7.5	6.5
Glucose Asparagine medium	55.2	Poor	--	5.2	5.5
Oat meal	120.3	Poor	--	6.5	5.5
Peptone Dextrose	55.8	Poor	Poor	5.3	5.8
Rose Bengal Agar medium	55.8	Poor	Fair	6.5	6.0
PCNB Agar modified	153.1	Fair	--	6.0	6.3
Potato Dextrose Agar medium	143.0	Excellent	--	6.5	6.0
Richard's medium	245.5	Excellent	--	5.4	6.9

Table : 14

Showing the average dry weight, sporulation, final pH and chlamydospore formation of *F. acuminatum* at different temperatures

S.No.	Temperature in °C	Dry wt. in mg.	Sporulation	Chlamydospore formation	Final pH
1.	5	--	--	--	--
2.	10	28.7	--	--	--
3.	15	53.9	Fair	Fair	5.4
4.	20	70.1	Excellent	Poor	7.4
5.	25	74.3	Excellent	--	7.5
6.	30	70.6	Excellent	Poor	7.4
7.	35	31.1	Fair	Fair	7.3
8.	40	--	--	--	--

Table : 15

Showing the average dry weight, sporulation, final pH and chlamydospore formation of *F. solani* at different temperatures

S.No.	Temperature in °C	Dry wt. in mg.	Sporulation	Chlamydospore formation	Final pH
1.	5	--	--	--	--
2.	10	28.9	Poor	Good	5.5
3.	15	41.5	Fair	Fair	7.0
4.	20	55.7	Excellent	Poor	7.3
5.	25	63.2	Excellent	Poor	7.3
6.	30	57.8	Excellent	Poor	7.3
7.	35	33.5	Fair	Good	7.1
8.	40	--	--	--	--

Table : 16

Showing the average dry weight, sporulation, final pH and chlamydospore formation of *F. oxysporum* at different temperatures

S.No.	Temperature in °C	Dry wt. in mg.	Sporulation	Chlamydospore formation	Final pH
1.	5	--	--	--	--
2.	10	20.9	--	Fair	5.3
3.	15	37.8	Poor	Poor	6.5
4.	20	50.1	Good	Poor	7.0
5.	25	58.0	Excellent	--	7.0
6.	30	51.3	Good	Poor	7.0
7.	35	19.7	Fair	Fair	6.5
8.	40	--	--	--	--

Table : 17

Showing the average dry weight, sporulation, final pH and chlamydospore formation of *F. equiseti* at different temperatures

S.No.	Temperature in °C	Dry wt. in mg.	Sporulation	Chlamydospore formation	Final pH
1.	5	--	--	--	--
2.	10	25.7	--	--	--
3.	15	50.8	Good	Good	5.3
4.	20	74.3	Excellent	Fair	7.5
5.	25	76.1	Excellent	Poor	7.4
6.	30	72.1	Excellent	Poor	7.5
7.	35	30.9	Fair	Fair	7.2
8.	40	--	--	--	--

Table : 18

Showing the average dry weight, sporulation, final pH and chlamydospore formation of *F. acuminatum* at various pH values

S.No.	pH of the medium	Dry wt. in mg.	Sporulation	Chlamydospore formation	Final pH
1.	2.0	--	--	--	--
2.	2.5	38.8	--	--	5.6
3.	3.0	47.2	Poor	--	6.7
4.	4.0	42.9	Poor	Poor	7.1
5.	4.5	56.1	Fair	Fair	7.8
6.	5.0	73.9	Excellent	Poor	7.3
7.	5.5	75.7	Excellent	--	7.5
8.	6.0	73.8	Excellent	Poor	7.5
9.	6.5	70.9	Excellent	Poor	7.5
10.	7.0	63.7	Good	Poor	7.7
11.	8.0	51.8	Fair	Fair	8.0
12.	9.0	40.3	Poor	Good	8.7
13.	10.5	38.1	Poor	Poor	8.5
14.	11.0	25.7	Poor	Poor	9.2

Table : 19

Showing the average dry weight, sporulation, final pH and chlamydospore formation of *F. oxysporum* at various pH values

S.No.	pH of the medium	Dry wt. in mg.	Sporulation	Chlamydospore formation	Final pH
1.	2.0	--	--	--	--
2.	2.5	30.1	--	Poor	5.1
3.	3.0	41.5	--	Fair	6.2
4.	4.0	54.7	Poor	Fair	6.7
5.	4.5	56.8	Fair	Poor	6.7
6.	5.0	56.9	Excellent	--	6.7
7.	5.5	58.3	Excellent	--	7.5
8.	6.0	52.3	Excellent	Poor	7.5
9.	6.5	52.7	Excellent	Poor	7.5
10.	7.0	42.3	Fair	Poor	7.7
11.	8.0	41.8	Fair	Poor	8.0
12.	9.0	35.0	Fair	Poor	7.8
13.	10.5	34.4	Fair	Poor	7.8
14.	11.0	21.2	Poor	Poor	8.2

Table : 20

Showing the average dry weight, sporulation, final pH and chlamydospore formation of *F. solani* at various pH values

Sl.No.	pH of the medium	Dry wt. in mg.	Sporulation	Chlamydospore formation	Final pH
1.	2.0	--	--	--	--
2.	2.5	38.7	--	Fair	5.2
3.	3.0	49.2	--	Fair	7.0
4.	4.0	50.1	Poor	Good	7.2
5.	4.5	52.7	Fair	Fair	7.5
6.	5.0	63.4	Excellent	Poor	7.8
7.	5.5	63.7	Excellent	--	7.8
8.	6.0	59.8	Excellent	Poor	7.8
9.	6.5	59.9	Good	Poor	8.0
10.	7.0	69.3	Fair	Poor	8.0
11.	8.0	51.2	Fair	Poor	8.2
12.	9.0	39.5	Poor	Good	8.2
13.	10.5	38.3	Poor	Poor	8.5
14.	11.0	28.6	Poor	Poor	8.8

Table : 21

Showing the average dry weight, sporulation, final pH and chlamydospore formation of *F. equiseti* at various pH values

S.No.	pH of the medium	Dry wt. in mg.	Sporulation	Chlamydospore formation	Final pH
1.	2.0	--	--	--	--
2.	2.5	--	--	--	--
3.	3.0	36.8	Poor	--	5.5
4.	4.0	43.7	Poor	Poor	6.5
5.	4.5	55.3	Fair	Fair	7.0
6.	5.0	70.0	Excellent	Poor	7.3
7.	5.5	75.9	Excellent	--	7.5
8.	6.0	75.7	Excellent	Poor	7.5
9.	6.5	72.5	Excellent	Poor	7.5
10.	7.0	65.7	Good	Poor	7.7
11.	8.0	57.8	Fair	Good	8.0
12.	9.0	48.0	Poor	Poor	8.5
13.	10.5	39.2	Poor	Poor	8.8
14.	11.0	20.5	Poor	Poor	9.0

Table : 22

Showing the average dry weight, sporulation, change in pH and Chlamydospore production by *Fusarium acuminatum* on different vitamins

Vitamins	Dry wt. in mg.	Sporulation	Final pH	Chlamydospore formation
Control	76.7	Excellent	7.4	Poor
All the six vitamins	84.2	Excellent	7.2	--
Ascorbic acid	68.0	Excellent	7.2	Poor
Biotin	84.1	Excellent	7.2	--
Folic acid	75.0	Excellent	7.5	--
Nicotinic acid	68.8	Excellent	7.0	Poor
Riboflavin	85.9	Excellent	7.5	--
Thiamine	92.9	Excellent	7.8	--

Table : 23

Showing the average dry weight, sporulation, change in pH and Chlamydospore production by *Fusarium oxysporum* on different vitamins

Vitamins	Dry wt. in mg.	Sporulation	Final pH	Chlamydospore formation
Control	40.0	Good	7.2	Poor
All the six vitamins	76.7	Excellent	7.2	--
Ascorbic acid	28.1	Good	7.0	Poor
Biotin	46.7	Good	7.5	Poor
Folic acid	48.3	Good	7.2	--
Nicotinic acid	29.8	Good	7.0	Poor
Riboflavin	55.2	Good	7.2	--
Thiamine	58.0	Excellent	6.8	--

Table : 24

Showing the average dry weight, sporulation, change in pH and Chlamydospore production by *Fusarium solani* on different vitamins

Vitamins	Dry wt. in mg.	Sporulation	Final pH	Chlamydospore formation
Control	60.0	Excellent	7.2	Poor
All the six vitamins	69.3	Excellent	7.5	--
Ascorbic acid	52.0	Excellent	7.1	Poor
Biotin	64.8	Good	7.5	--
Folic acid	67.8	Excellent	7.5	--
Nicotinic acid	75.7	Good	7.5	--
Riboflavin	67.9	Good	7.5	--
Thiamine	47.3	Good	7.5	Poor

Table : 25

Showing the average dry weight, sporulation, change in pH and Chlamydospore production by *Fusarium equiseti* on different vitamins

Vitamins	Dry wt. in mg.	Sporulation	Final pH	Chlamydospore formation
Control	68.0	Excellent	7.0	Poor
All the six vitamins	77.5	Excellent	7.3	--
Ascorbic acid	50.0	Good	7.0	--
Biotin	65.3	Good	7.5	--
Folic acid	68.9	Excellent	7.5	--
Nicotinic acid	78.7	Excellent	7.5	Poor
Riboflavin	68.7	Excellent	7.5	--
Thiamine	43.5	Good	7.5	--

Chapter 5

Control Studies

Healthy Crops of Arhar (*Cajanus cajan*)

CONTROL STUDIES

Plant disease control methods, a vary considerably from one disease to another, depending on the kind of pathogen, the host and the interaction of the two. In controlling diseases, plants are generally treated as populations rather than as individuals, although certain hosts (specially trees, ornamentals and some times virus infected plants) may be treated individually.

The various control methods can be classified as regulatory, cultural biological, physical, and chemical depending on the nature of the agents employed. Regulatory control measures aim at excluding a pathogen from a host or from a certain geographic area. Most cultural control methods aim at helping plants avoid contact with a pathogen and at eradicating or reducing the amount of pathogen in a plant, a field or an area. Biological and some cultural control methods aim at improving the resistance of the host. Finally physical and chemical methods aim at protecting the plants from pathogen inoculum that is likely to arrive and at curing on infection that is already in progress.

The word fungicides has originated from two latin words viz., fungus and caedo. The words caedo means "to kill". Thus a fungicide could be any agencies which has the ability to kill a fungus. The fungicides are just one of the weapons in our essential of defence against unwanted diseases of plant. Fungicides may

work alone or may be used in combination to prevent plant diseases. Once a disease becomes established in field it is generally difficult to eliminate.

Workers including Valasoka (1962), Sen Gupta and Roy (1971) and Ilyas *et al.*, (1975) have tested a number of fungicides for soil disinfection. In view of this fact, a number of fungicides were tried to check the population of *Fusarium* sp. in soil.

Hasan & Khan (1979, 1980) observed the fungicidal control of anthracnose diseases (*Colletotrichum trancatum*) of Arhar (*Cajanus cajan*). Paharia (1983) observed the pest and diseases problems on rice, gram (*Cicer arietinum*), arhar (*Cajanus cajan*), lentil, mustard (*Brassica, jancea*) soybean and sugarcane in Madhya Pradesh, India. Bist, Kannaiyan and Nene (1988) applied three methods of application of Metalaxyl (Ridomil) seed dressing (SE) soil application (SQ) and spray were tested separately and in combination for the control of phytophthora blight in pigeonpea caused by *Phytophthora drechsleri cajani*.

In preliminary test, the efficiency of a number of fungicides viz., Dithan M-45 (Mancozeb), Vorlan (Vinclozolin), Ridomil (Acylalanine), Benlate (Benomyl), Topsin (Thiophanate), Topsin M, (Thiophanate methyl), Plantvax (Oxycarboxin), Hinoson (Edifenphos) against four species of *Fusarium* was tried *in vitro*. The fungicides were added to the basal medium at two concentrations (100 ppm and 500 ppm) and flasks were steam sterilized for 3 successive days for 30 minutes.

All the fungicides were amended in the soil at a concentration which was found to be inhibitory to the growth of the organisms in culture media. The fungicides were amended to the soil at a concentration of 100 and 500 ppm. The fungal inoculum was incorporated in the soil after 24 hours of addition of the fungicides. The results are summarized in the Table 26.

Results show that Topsin and Topsin M were found to have a marked retarding effect on the survival of all the four species of *Fusarium*. At a concentration of 100 and 500 ppm both, the fungal survival was almost eliminated. The incidence of survival of four species of *Fusarium* was slightly increased in case of Ridomil and Hinoson respectively as compaired to other fungicides. Dithan M-45, Vorlan, Plantvax were less effective than Benlate in reducing the population of present species of *Fusarium* at 100 and 500 ppm. But all fungicides were some effective than control for reducing the population, *Fusarium solani, F. oxysporum, F. equiseti* and *F. acuminatum*. Similar results were obtained by Ilyas *et al.*, (1976) for *Macrophomina phaseolina* and Misra, (1992) for three species of *Fusarium*.

Seed treatment either with fungicides or biological agents prior to sowing in general believed to be a profitable practice because it eliminated or reduced seed-borne pathogens and raised seed germination, seedling emergence and vigour. Treatment of pigeonpea seeds with fungicides either eliminated or reduced the number of colonies of the pathogens associated with seeds (Table 27). Out of

eleven fungicides evaluated, Bavistin-50 WP, Bavistin 25 SD + Thiram, Bavistin 25 SD and Captan were found to eliminate the entire mycoflora associated with pigeonpea seeds followed by Vitavax in which all the fungal species were eliminated except few colonies of *Fusaria*. The remaining fungicides, viz. Thiram, Agrosan GN, Dithane M-45, Captafol and Kavach are listed in descending order so far as their effectiveness in elimination of pathogens was concerned. Seed treatment with Brassicol was however, found least effective. The data on seed germination and seedling emergence were recorded after 10 and 21 days of sowing, respectively, analysed statistically and are summarized in Table 28.

The results of Table 28 reveal that fungicidal seed treatment generally improve the seed germination, seedling emergence and vigour. Out of eleven frungicides tried, Bavistin 50 WP and Bavistin 25 SD + Thiram though not significantly different themselves but were found superior to others and control. Captan, Bavistin 25 SD, Vitavax, Dithane M-45 and Captafol were found next in their effectiveness. Brassicol, Thiram, Agrosan GN and Kavach treated seeds gave no significant inhancement in either germination or seedling emergence. However, seedling vigour as adjudged on the basis of shoot and root length, and fresh weight was significantly improved in all the fungicides over control. The shoot length of the seedling from treated seeds was significantly increased in all the fungicides except Brassicol. Captan seed treatment induced maximum increase in root length and seedling fresh weight while the root length was found maximum in the

seedlings, which developed from Thiram treated seeds, followed by Bavistin 25 SD and Bavistin 25 SD + Thiram. Kavach, Captafol and Agrosan GN treated seed gave, non-significant response in raising seedling vigour.

Pranab Dutta and B.C. Das, (2002) reported that the preparation of *Trichoderma* sp. is effective in controlling the collor-rot of tomato incited by *Sclerotium rolfsii* and reduced saprophytic growth of the pathogen.

Chaetomium globosum and *Trichoderma viride* the known antagonists were tested for their efficacy in controlling the seed-borne pathogens of pigeonpea as described under 'Materials and Methods'. The results are summarized in Table 29.

A perusual of Table 29 shows that seed treatment with *T. viride* and *C. globosum* were effective in eliminating or reducing the pathogens associated with the seeds. *T. viride* treated seeds eliminated entire pathogenic flora except *A. flavus, A. niger, B. cinerea, F. moniliforme* and *F. semitectum*, whereas *C. globosum* treated seeds reduced the incidence of pathogens. It did not eliminate the infection of pathogens except *Cladosporium cladosporioides* which was completely checked. However, both the antagonists were found to reduce the incidence of each and every pathogen.

In order to see the effect of *C. globosum* and *T. viride* on germination, emergence and vigour of seedlings, both laboratory and field experiments were

carried out as described under 'Materials and Methods'. The data are summarized after statistical analysis in the Table 30.

It is evident from the results of Table 30, that *T. viride* treated seeds significantly increased seed germination, emergence and vigour of pigeonpea seedlings. The increased percentage of seedling emergence and improved shoot length, root length and fresh weight of the seedlings in *T. viride* treated seeds indicated that this treatment not only eliminated the pathogens responsible for causing seed rot and seedling mortality but also produced vigourous seedlings. On the other hand, *C. globosum* treatment was though effective in reducing the number of colonies of pathogens and improving seed germination, seedling emergence and vigour but not significantly enough.

In this experiment, two fungal species viz. *Chaetomium globosum* and *Trichoderma viride* which were earlier considered as antagonists (Selvarajan, 1990) were applied to the seeds for observing their role in controlling the pathogens associated with chickpea seeds as well as in improving the seed germination, seedling emergence and vigour. *C. globosum* was isolated from pigeon pea seeds of Pant 8508 and other one, *T. viride* was obtained from Herbarium Cryptogame Indie Orientalis, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi-12, India..

Germination of fungal spores is essentially a process during which the normal metabolic and physiological activity is restored after dormancy. According to Gottlieb (1964) "Germination is the process by which a spore is transferred from a dormant state of low metabolic activity to one of high metabolic activity. Formation of the germ tube is the out word visible sign that the metabolic change is complete.

Spores are known to be more sensitive to environment than mycelium hence it was considered necessary to investigate the effect of leaf extracts of some medicinal plants on the germination of spores of present fungi at room temperature (25 ± 1°C). Seven days old culture of the four *fusarial* pathogens viz. *F. solani, F. oxysporum, F. acuminatum* and *F. equiseti* have been used. Leaves of several plants have been reported to posses chemicals, toxic to various micro-organisms and serve as chemical protective barrier to the infection.

Anti-fungal property of several plant extracts has been reported by various workers (Sekhawat and Prasad, 1971; Misra *et al.*, 1974 and Misra, 1975). Present work deals with the effect of leaf extracts of a number of medicinal plant on spore germination of the fungi under study. Spore suspensions were made in the supernatant extract at two concentrations viz., 100% and 50% and the percentage germination of the spores was recorded after initial time of germination in the Table 31.

It is evident from the table 31 that out of nine medicinal plant tried leaf extract of Neem (*Azadirachta indica*) at 100% concentration completely checked the spore germination of all the four fungi, while in 100% extract of *Ocimum sanctum* L. spore germination of all four *Fusarium* sp. below 20%. Next 100% concentration of *Nicotiana plumbagiuifolia* and *Achyranthus aspera, Argemone maxicana* extracts of spore germination were 20% but extracts of Datura and *Lantana camera* on germination of all four *Fusarium* sp. no significant results (near about 50% germination both concentration 50% and 100%). In comparison to the above leaf-extracts spore germination of *F. oxysporum, F. solani, F. equiseti* and *F. acuminatum* were 70, 88, 90 and 86 respectively in control. Leaf-extract of some medicinal plants which were found effective during spore germination were also tried controlling the wilt of plants at the seedlings by Mishra, (1992), Srivastava, (1996) and Shukla, (2000).

Different workers investigated the effect of leaf-extracts of various medicinal plants on spore germination of pathogenic fungi. Sekhawat and Prasad (1971) tried leaf-extract of *Melia azadirachta, Ocimum sanctum* and *Allium sativum* against 41 species of pathogenic fungi out of which *Curvularia penniseti* and *Helminthosporium* sp. were found unable to germinate on *Melia* and *Ocimum* leaf-extracts. Misra *et al.,* (1974) reported complete inhibition of spore germination of *Curvularia lunata* and *Helminthosporium graminicola* in leaf extracts of *Melia* and *Ocimum* respectively. Khanna and Chandra, (1972) have also reported similar observations for the fungi studied by them.

S.No.	Fungicides	Dose (g/kg seed)	Active ingradients	Group of fungicides
I. Systemic Fungicides				
1.	Bavistin 25 SD	2.0	25% 2 (Methoxy-carbamoyl) - benzimidazole	Benzimidazole
2.	Bavistin 50 WP	2.0	50% 2 (Methoxy-carbamoyl) - benzimidazole	-- do --
3.	Vitavax	2.0	75% 2, 3 dihydro-5-carboxanilido, 6-methyl-1, 4-oxathin	Oxathin
II. Systemic + Non-systemic Fungicide				
4.	Bavistin 25 SD + Thiram	1.0 + 1.0	25% 2-(Methoxy-carbamoyl) - benzimidaxole + 80% Tetramethyl thiuram disulphide	Benzimidazole + Organic sulphur
III. Non-systemic Fungicides				
5.	Agrosan GN	3.0	1% Mercury in Tolymercury acetate	Organic mercury
6.	Brassicol	2.0	75% Pentachloronitro-benzene	Benzene
7.	Captafol	2.0	80% Cis-N (1, 1, 2, 2-tetra chloroethylthio)-4 cyclohexane-1, 2-dicarboximide	Heterocyclic nitrogen
8.	Captan	2.0	75% N-trichloro-methyl-thio-4-cyclohexane-1, 2-dicarboximide	-- do --
9.	Dithane M-45	2.0	80% Zinc (2%) + 78% Mangneese ethylene-bis-dithiocarbamate	Organic sulphur
10.	Thiram	2.0	80% Tetramethyl thiuram disulphide	-- do --
11.	Kavach	2.0	75% Tetrachloro-isoph-thalonitrile	Miscellaneous

Effect of Neem products on seed mycoflora of pigeonpea

Neem (Azadirachta indica) belongs to family Meliaceae is a native of Indian subcontinent is a highly stemmed tree for the people in the region. This tree has been considered so invaluable and miraculous that it becomes a major inseparable component of the Indian ecosystem. Over 300 inside large number of nematode and plant pathogens are already reported to be controlled various neem products.

The variety of Arhar plant was "UPAS-120". The treatments with 4 replication were allotted in a random manner. The treatment were designated as T_o, T_1, T_2, T_3, T_4, T_5. Where T_o (Control), T_1 (Neem bark), T_2 (Neem cake), T_3 (Neem leaf), T_4 (Neemoria), and T_5 (Neem shield).

(a) Observation on plant growth parameters are recorded at 30 DAS, 60 DAS, 90 DAS were recorded.

(b) On Mycoflora population observation were recorded at 30 DAS, 60 DAS, 90 DAS and also presowing stage.

All glass wares clean thoroughly with the help of vim powder and the wash clean water, then dried in shade and wrapped with paper and then sterilized in Oven at a temperature of 160°C for two hours. Water blanks were prepared by filling desired quantity (100 ml and 90 ml) of water into clean conical flasks then

plugging with cotton plug, then these conical flasks with water was autoclaved at 15 lbs/inch^2 for 15 minutes. Soil sample were collected at the presowing time at five places as each replication and mixed well and 10 gm soil collected was transferred into conical flask containing 100 ml sterilized water.

For soil analysis, one plant from each replication were gently lifted up from soil. The excess soil was shaken off the roots by gently tapping the stem. The plants are taken at random with the help of sterilized scissors the root system was cut from the plant and transferred containing 500 ml sterilized water blank and quickly replaced the cotton plug care should be taken not to touch the root with the hand at any stage.

The effect of different neem products on rhizosphere mycoflora was studied and data were recorded at presowing stage, 30 DAS, 60 DAS, 90 DAS. The predominant fungal genera observed in the rhizosphere soil were *Fusarium oxysporum*, *Mucor* sp., *Rhizopus* sp., *Alternaria* sp., *Pencillium* sp., *Aspergillus* sp.

The distribution of fungal population of individual fungal genera at presowing stage 30 DAS, 60 DAS, 90 DAS with neem products including control (T_o) is presented in table no. 32-35 respectively.

The percentage of genera of fungi encountered in all the stages are shown in decreasing order as follows presowing stage table no. 32 the predominance

fungi was order. *Fusarium oxysporum* (23.68) > *Mucor* sp. (17.30) > *Rhizopus* sp. (16.35) > *Pencillium* sp. (15.41) > *Aspergillus niger* (15.15) > *Alternaria* sp. (11.23).

30 DAS : Showing Table No. 33.

T_o : The predominance fungi was in order. *Penicillium* sp. (21.67) > *Fusarium oxysporum* (20.23) > *Alternaria* sp. (18.20) > *Aspergillus niger* (17.19) > *Mucor* sp. (13.29) > *Rhizopus* sp. (9.39).

T_1 : *Penicillium* sp. (21.60) > *Fusarium oxysporum* (20.48) > *Alternaria* sp. (18.20) > *Aspergillus niger* (17.19) > *Mucor* sp. (13.22) > *Rhizopus* sp. (9.31).

T_2 : *Penicillium* sp. (21.47) > *Fusarium oxysporum* (20.44) > *Alternaria* sp. (18.20) > *Aspergillus niger* (17.17) > *Mucor* sp. (13.29) > *Rhizopus* sp. (9.40).

T_3 : *Penicillium* sp. (21.44) > *Fusarium oxysporum* (20.33) > *Alternaria* sp. (18.10) > *Aspergillus niger* (17.13) > *Mucor* sp. (13.23) > *Rhizopus* sp. (9.33).

T_4 : *Penicillium* sp. (29.33) > *Fusarium oxysporum* (25.00) > *Aspergillus niger* (20.98) > *Mucor* sp. (16.29) > *Rhizopus* sp. (11.38).

T_5 : *Penicillium* sp. (27.73) > *Aspergillus niger* (25.69) *Fusarium oxysporum* (21.88) > *Mucor* sp. (19.83) > *Alternaria* sp. (4.83).

Population density of various Rhizosphere fungi per gram of dry soil in (10^3).

60 DAS : Showing Table No. 34

T_o : The predominance fungi was in order. *Aspergillus niger* (22.04) > *Penicillium* sp. (21.23) > *Fusarium* oxysporum (20.45) > *Mucor* sp. (18.86) > *Rhizopus* sp. (17.27).

T_1 : *Alternaria* sp. (26.59) > *Penicillium* sp. (23.40) > *Aspergillus niger* (22.74) > *Mucor* sp. (14.57) > *Fusarium oxysporum* (12.56).

T_2 : *Aspergillus niger* (20.09) > *Rhizopus* sp. (19.78) > *Penicillium* sp. (15.77) > *Fusarium oxysporum* (15.16) > *Mucor* sp. (13.93) = *Alternaria* sp. (13.93).

T_3 : *Aspergillus niger* (25.00) > *Mucor* sp. (21.26) > *Rhizopus* sp. (19.15) > *Alternaria* sp. (17.52) > *Fusarium oxysporum* (16.56).

T_4 : *Aspergillus niger* (18.44) > *Penicillium* sp. (17.75) > *Fusarium oxysporum* (17.24) > *Mucor* sp. (15.86) = *Alternaria* sp. (15.86) > *Rhizopus* sp. (14.48).

T_5 : *Aspergillus niger* (27.90) > *Mucor* sp. (26.79) > *Fusarium oxysporum* (23.23) > *Rhizopus* sp. (21.81).

90 DAS : Showing Table No. 35.

Population density of various Rhizosphere fungi per gram of dry soil in (10^3).

T_o : *Penicillium* sp. (23..02) > *Aspergillus niger* (22.54) = Mucor sp. (22.54) > *Fusarium* oxysporum (17.50) > *Rhizopus* sp. (13.66).

T_1 : *Aspergillus niger* (27.65) > *Fusarium oxysporum* (27.00) > *Mucor* sp. (23.47) > *Penicillium* sp. (21.54) .

T_2 : *Aspergillus niger* (29.94) > *Mucor* sp. (29.36) > *Fusarium oxysporum* (22.67) > *Penicillium* sp. (17.73).

T_3 : *Aspergillus niger* (30.60) > *Fusarium oxysporum* (24.86) > *Mucor* sp. (22.95) > *Penicillium* sp. (21.03).

T_4 : *Mucor* sp. (31.20) > *Aspergillus niger* (25.53) > *Fusarium oxysporum* (24.10) > *Penicillium* sp. (18.85).

T_5 : *Aspergillus niger* (30.74) > *Fusarium oxysporum* (25.00) > *Mucor* sp. (22.96) > *Penicillium* sp. (21.28).

Treatment (T_5) was significant difference among the treatment and treatments (T_4 & T_2) and (T_2 & T_3, T_1) with (T_1 & T_0) were not significantly difference each other and were in order $T_5 > T_4 > T_2\ T_3 > T_1 > T_0$.

The different neem products as Neem bark (T_1), Neem cake (T_2), Neem leaf (T_3), Neemoria (T_4), Neem shield (T_5) inhibited the growth of fungal populations compared to presowing stage than 30 DAS, 60 DAS and 90 DAS.

There was decrease in fungal population at 30 DAS in all treatments except Neem leaf (T_3) compared to control. At 60 DAS Neem leaf (T_3) and Neem shield (T_5) were effective in controlling fungal populations compare to control. At 90 DAS all treatments except (T_1) neemoria reduced the fungal population compare to control (T_o). The result obtained in our study are in general agreement with reports of Singh & Singh (1983).

Neem shield give more effective results than other neem products at all stages of plant growth. Considering above observation it is concluded that the results given by neem shield was best in management of rhizosphere mycoflora giving better plant growth as well.

RESISTANCE VARIETIES

The use of disease resistant varieties for controlling plant diseases has been termed as the "Painless method" because it does not cost the farmers anything. In an under developed country like India, it is all the more important since we cannot pay for the heavy costs of spraying and dusting crops on a large scale use of dangerous fungicides and pesticides. It also reduces pollution which results from the use of poisonous chemicals and their residues. Resistant crop varieties check epidemics of pathogens and pests and thus help to maintain the biological balance in the environment.

The resistant plant defends itself against a potential pathogen by means of a number of a physical and chemical characteristics of the plant which are formed in the plant in response to infection. The physical characteristics act as chemical barriers which prevent the entrance and spread of pathogen in the plant. The chemical factors, which are toxic to the pathogen inhibit its growth and activity in the plant.

Since Biffen's, (1905) elucidation of the inheritance of the resistance in singal Mendelian fashion, spectacular progress has been made in our understanding of the genetic aspects of parasitism and disease resistance. The mechanism of variability that make the pathogens versatile in their behaviour and host range are now well known.

Flor, (1955) explained host parasite interaction, inflax rust by assuming gene for gene-relationship between rust reaction in the host and pathogenicity in the parasite. He gave a hypothesis 'gene for gene' to explain the genetic basis of host specificity.

Hort, (1926) observed that the stomata of rust-resistant wheat remain closed till late in the morning. By the time they open, the germ tubes of uredospores, formed earlier in the dew, get killed due to evaporation of the water.

Link and Walker, (1933) reported presence of protocatecuic acid and catechol in the dry pigmented scales of onion bulbs resistant to *Colletotrichum circinans*. Timonin, (1940) reported that resistant varieties of flax excrete hydrocyanic acid (HCN) in the the rhizosphere Orton, (1900) obtained resistant cotton variety from selection and multiplication of resistant individuals. He observed that some cotton plants did not show wilting in the heavily infected crop. He collected seeds from these plants and planted them in wilt infested soil. By several such plantings he ultimately got most resistant plants which grew well on heavily infested soils. A vast majority of crop varieties can be attacked by a single pathogen or many different kinds of pathogen. Most plants are naturally resistant to many pathogen.

Rajkule, Goyal and Vala, (1989) observed on different entries revealed that in both seasons wilt first appeared around 50 days after sowing continuing and

increasing upto maturity in indicator rows (ICP 2376) was upto 94% in 1986 and 63% in 1987 of the test entries, the only one observed to be totally wilt free in both seasons, was BP1809. Four entries (BDN2, BP1314, BP2094, and BP2061) showed less than 10% mortality the variety Nylon recorded more than 70% mortality.

Mukherjee, De and Parui (1971), obtained 58 varieties of *Cajanus cajan* screened for resistance to *Fusarium udum* by a technique which is described, none was resistant but nine were moderately resistant. Evidence was found for the existence of pathogen races. The technique allows for the identification of races on the basis of reaction to a set of differential varieties.

Hasan & Khan, (1979) obtained 49 Arhar (Pigeonpea) cultivars were screened against *Colletotrichum truncatum* some proved highly susceptible, some were resistant and a few (NPWR-015, PANT A9, Prabhat, R-98 and 1234) were immune. When planted late (September-October) a few of the susceptible cvs. escaped sever disease.

Singh *et al.*, (1988) observed "Pusa-74" a high yielding variety. Derived from the cross Khargone 2 x Pusa Ageti and released for cultivation in Maharastra, Gujarat, Madhya Pradesh and Rajasthan in 1982, the *Cajanus cajan* v. Pusa 74 averaged 17.84% higher yield than the control T21, over the years 1974 to 1981. Both are in the 150-day maturity group. Comparative data on annual yield in the

trial years and on 8 yield related traits are tabulated. Pusa 74 is tolerant of infection by *Fusarium udum* and *Macrophomina phaseolina* and relative tolerant *Phytophthora drechsleri, Fusarium* sp. *cajani* and sterility mosaic virus.

Singh, (1998) observed Narendra Arhar 1, along duration, high yielding Pigeonpea (*Cajanus cajan*) cultivar with resistance to tolerance of important biotic and abiotic stresses, was developed through pedigree selection from a local collection from Faizabad district. It is easily distinguished from existing cultivars on the basis of its growth habit, flowers and pod characteristics. Narendra Arhar-1 produces brown seeds with 100 seed weight of about 11 gm.

Pathogenicity tests were performed by planting healthy seedlings in pots containing infested soil with 3% maize meal inoculum. The results are summarized in Table 36.

Results from the above experiments clearly show that out of ten varieties of Arhar (*Cajanus cajan*) were tested against *Fusarium oxysporum, F. solani, Fusarium equiseti* and *F. acuminatum.* Only NP (WR) 15, Sharda, ICP8863 (Maruthi) and Pusa-9 were completely resistant to the pathogen under investigation. Mukta was resistant against *F. oxysporum, F. equiseti, F. solani*, but moderately resistant for *F. acuminatum*. Cultivar C 11 was completely resistant against *F. oxysporum* and *F. acuminatum* but moderately resistant for *F. solani* and *F. equiseti*. BDN2 show resistant against *F. oxysporum, F. equiseti* and moderately resistant for *F. solani* but susceptible for *F. acuminatum*. Cultivars Bahar, BDN1 and TT6 were susceptible against four *Fusarium* specieses.

Table : 26 : Effect of fungicides amendment in soil on *Fusarium* species population

Fungicides	Concentration (ppm)	Number of colonies per 50 mg. of soil			
		Fusarium solani	*F. oxysporum*	*F. acuminatum*	*F. equiseti*
Dithane M-45	100	41	30	37	16
	500	48	20	22	14
Vorlan	100	46	35	31	20
	500	39	23	22	25
Ridomil	100	52	43	35	32
	500	38	37	27	21
Benlate	100	15	18	13	11
	500	04	03	05	00
Topsin	100	00	00	02	01
	500	00	00	00	00
Topsin M	100	00	00	00	02
	500	00	00	00	00
Plantvax	100	35	31	42	50
	500	29	25	21	19
Hinoson	100	51	47	36	43
	500	40	36	31	27
Control		67	53	48	49

Table : 27 : Percent seed infection of different pathogens found associated with fungicides treated and untreated pigeonpea seeds (Number of seeds tested : 200)

Fungi	A	B	C	D	E	F	G	H	I	J	K	L
Alternaria alternata	16	6	8	3	2	-	1	-	-	-	-	-
A. longissima	6	3	4	--	--	--	1	--	--	--	--	--
Aspergillus flavus	12	9	1	--	--	--	--	--	--	--	--	--
A. niger	6	4	--	--	--	--	--	--	--	--	--	--
Botrytis cinerea	2	1	--	--	--	--	--	--	--	--	--	--
Cladosporium cladosporioides	5	--	--	--	--	--	--	--	--	--	--	--
Colletotrichum dematium	6	4	3	1	--	1	--	--	--	--	--	--
Curvularia lunata	5	3	3	2	--	--	--	--	--	--	--	--
Fusarium moniliforme	11	4	6	6	5	4	3	4	--	--	--	--
F. semitectum	8	5	5	4	3	2	2	3	--	--	--	--
Phyllosticta cajani	7	3	--	1	--	--	--	--	--	--	--	--
Rhizoctonia bataticola	9	--	5	--	1	4	1	1	--	--	--	--
R. solani	6	--	3	--	1	1	--	--	--	--	--	--
Trichothecium roseum	8	1	3	--	--	--	--	--	--	--	--	--
Total number of pathogens detected	14	11	10	6	5	5	5	3	--	--	--	--
Total number of fungul colonies	107	43	41	17	12	12	8	8	--	--	--	--

A- Control B-Brassicol C-Kavach D-Captafol E-Agrosan GN F-Dithane M-45 G-Thiram H-Vitavax

I-Bavistin 50 WP J-Bavistin 25 SD K-Bavistin 25 SD + Thiram L-Captan

Table : 28 : Effect of fungicidal seed treatment on seed germination, seedling emergence and vigour of pigeonpea

Fungicides	Dose (g/kg seed)	Seed germination		Seedling emergence	Seedling vigour		
		Under laboratory conditions	In pot		Shoot length (mm)	Root length (mm)	Seedling fress weight (mg)
		Av. transformed angular values	Av. transformed angular values	Av. transformed angular values			
Bavistin 50 WP	2.0	69.86 (88.14)	66.59 (84.21)	54.24 (81.11)	91.4	86.0	2828.75
Bavistin 25 SD + Thiram	1.0+1.0	67.37 (85.19)	63.48 (80.06)	62.86 (79.19)	94.1	73.8	2851.25
Captan	2.0	64.23 (81.09)	62.09 (78.08)	60.04 (75.06)	88.5	90.9	3022.50
Bavistin 25 SD	2.0	64.96 (82.08)	62.06 (78.04)	61.46 (77.17)	94.3	85.1	2662.50
Vitavax	2.0	63.48 (80.06)	61.39 (77.07)	59.41 (74.10)	83.3	76.2	2654.50
Captafol	2.0	63.51 (80.10)	60.72 (76.08)	59.38 (74.06)	83.5	81.2	2575.75
Dithane M-45	2.0	62.77 (79.05)	60.71 (76.06)	59.34 (73.99)	93.8	75.0	3018.75
Thiram	2.0	61.40 (77.08)	59.43 (74.13)	56.83 (70.07)	99.5	77.2	2768.75
Agrosan GN	3.0	60.82 (75.25)	59.42 (74.11)	56.20 (69.05)	81.9	86.1	2557.50
Kavach	2.0	60.75 (76.12)	58.12 (72.10)	56.82 (70.05)	74.5	68.3	2526.25
Brassicol	2.0	59.38 (74.05)	58.08 (72.04)	55.00 (67.10)	62.0	74.1	2775.25
Control	--	57.46 (71.07)	56.24 (69.12)	51.99 (62.08)	59.6	58.9	2102.50
CD (= 0.05)		4.08	4.23	5.11	5.61	5.98	515.12

Table : 29

Percent seed infection of different pathogens found associated with pigeonpea seeds treated with antagonists (Number of seeds tested : 200)

Fungi	Control	*Chaetomium globosum*	*Trichoderma viride*
Alternaria alternata	16	08	--
A. longissima	03	01	--
Aspergillus flavus	11	05	08
A. niger	04	01	02
Botrytis cinerea	01	01	01
Cladosporium cladosporioides	02	--	--
Colletotrichum dematium	04	03	--
Curvularia lunata	03	01	--
Fusarium moniliforme	10	09	07
F. semitectum	05	02	01
Phyllosticta cajani	06	04	--
Rhizoctonia bataticola	08	07	--
R. solani	05	03	--
Trichothecium roseum	04	04	--

Percent seed infection of different pathogens found associated with pigeonpea seeds treated with antagonists (Number of seeds tested : 200)

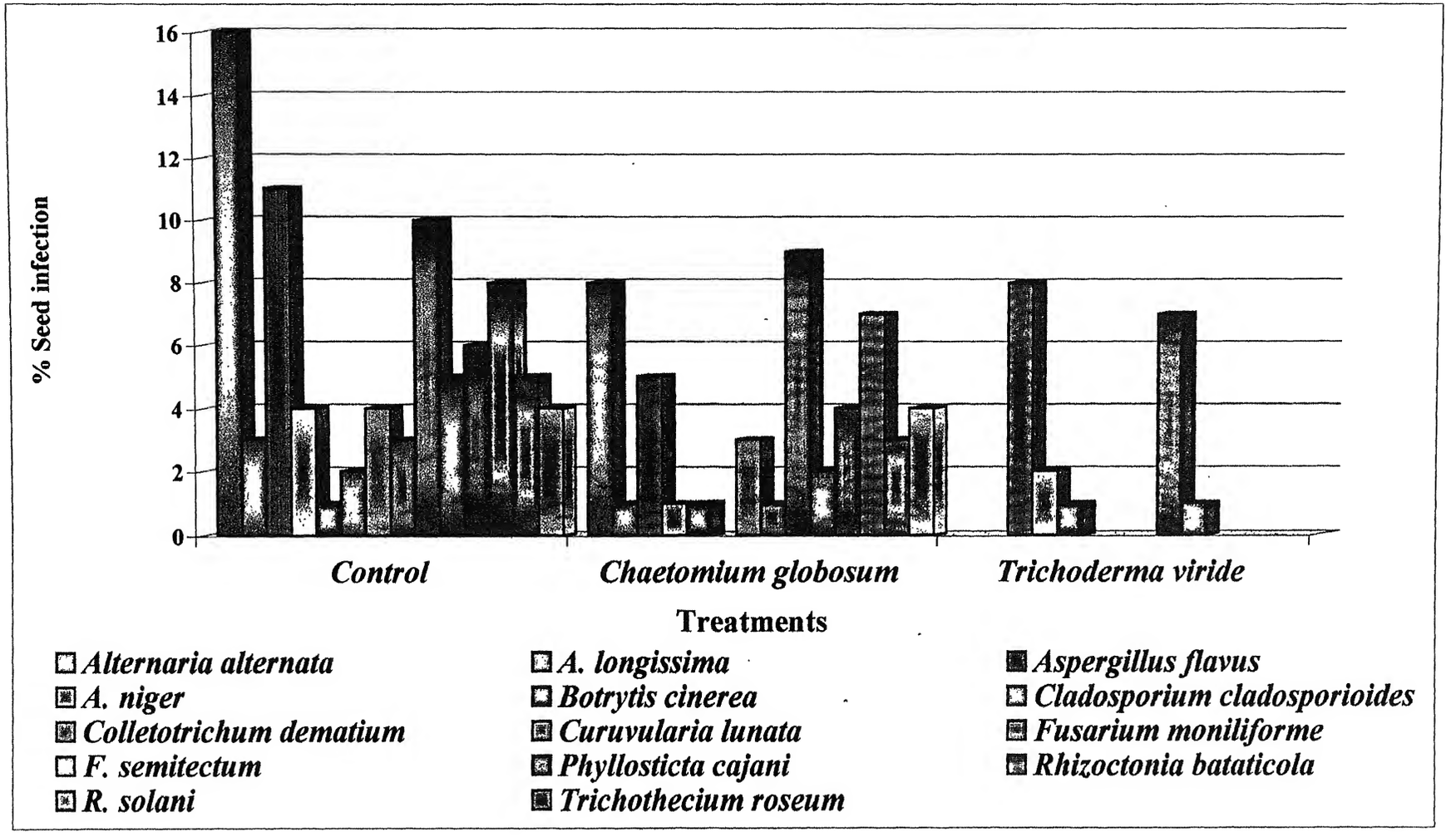


Table : 30 : Effect of biological seed treatment on seed germination, seedling emergence and vigour of pigeonpea

Antagonists	Dose (g/kg seed)	Seed germination		Seedling emergence	Seedling vigour		
		Under laboratory conditions	In pot		Shoot length (mm)	Root length (mm)	Seedling fress weight (mg)
		Av. transformed angular values	Av. transformed angular values	Av. transformed angular values			
Tricoderma viride	2.0	62.07 (78.06)	59.39 (74.07)	60.72 (76.08)	89.4	84.5	2907.75
Chaetomium globosum	2.0	57.44 (71.03)	55.57 (68.03)	53.75 (65.03)	63.8	66.7	2283.00
Control	--	54.69 (67.04)	53.80 (65.12)	52.01 (62.11)	61.3	64.5	2135.50

Table : 31

Showing effect of leaf-extracts of some plants on spore germination of *Fusarim* sp.

Leaf-extracts	Concentration %	Spore germination in percentage			
		F. oxysporum	*F. solani*	*F. equiseti*	*F. acuminatum*
Ocimum sanctum	50%	27	30	25	23
	100%	18	20	17	19
Azadirachta indica	50%	11	08	10	07
	100%	--	--	--	--
Argemone mexicana L.	50%	35	41	38	39
	100%	23	19	25	24
Colotropis procera	50%	45	52	48	46
	100%	27	25	23	21
Strychnus nuxvomica	50%	43	48	41	39
	100%	26	30	33	35
Datura fastuosa L.	50%	46	55	53	52
	100%	40	48	49	51
Achyranthus aspera L.	50%	37	35	33	50
	100%	20	26	23	21
Nicotiana plumbagiuifolia	50%	29	25	32	33
	100%	20	19	22	25
Lantana camera	50%	52	57	60	59
	100%	51	54	59	63
Control (Distilled water)	--	70	88	90	86

Table 32 : Population density of various fungi per gram of dry soil (10^3) at presowing stage.

Organisms	Replications									
	R_1	%	R_2	%	R_3	%	R_4	%	Means	%
Fusarium oxysporum	1.75	22.17	0.64	9.23	1.23	19.52	1.52	18.31	1.285	23.68
Mucor sp.	1.4	17.78	0.96	13.65	0.60	6.82	1.69	20.36	1.165	17.30
Rhizopus sp.	0.87	11.02	1.77	25.41	0.92	14.60	1.35	16.26	1.227	16.35
Penicillium sp.	1.57	19.89	1.61	23.23	1.84	29.20	1.86	22.40	1.720	15.41
Aspergillus niger	1.22	15.46	1.45	20.92	0.76	12.06	1.01	12.16	1.110	15.15
Alternaria sp.	1.05	13.30	0.48	6.92	0.92	14.60	0.82	10.12	0.820	11.23
Total	7.86	100	6.91	100	6.27	100	8.25	100	7.33	100

Variance (F-value) at 5%, due to replications - Non-significant
due to treatments - Non-significant

Population density of various fungi per gram of dry soil (10^3) at pre-sowing stage

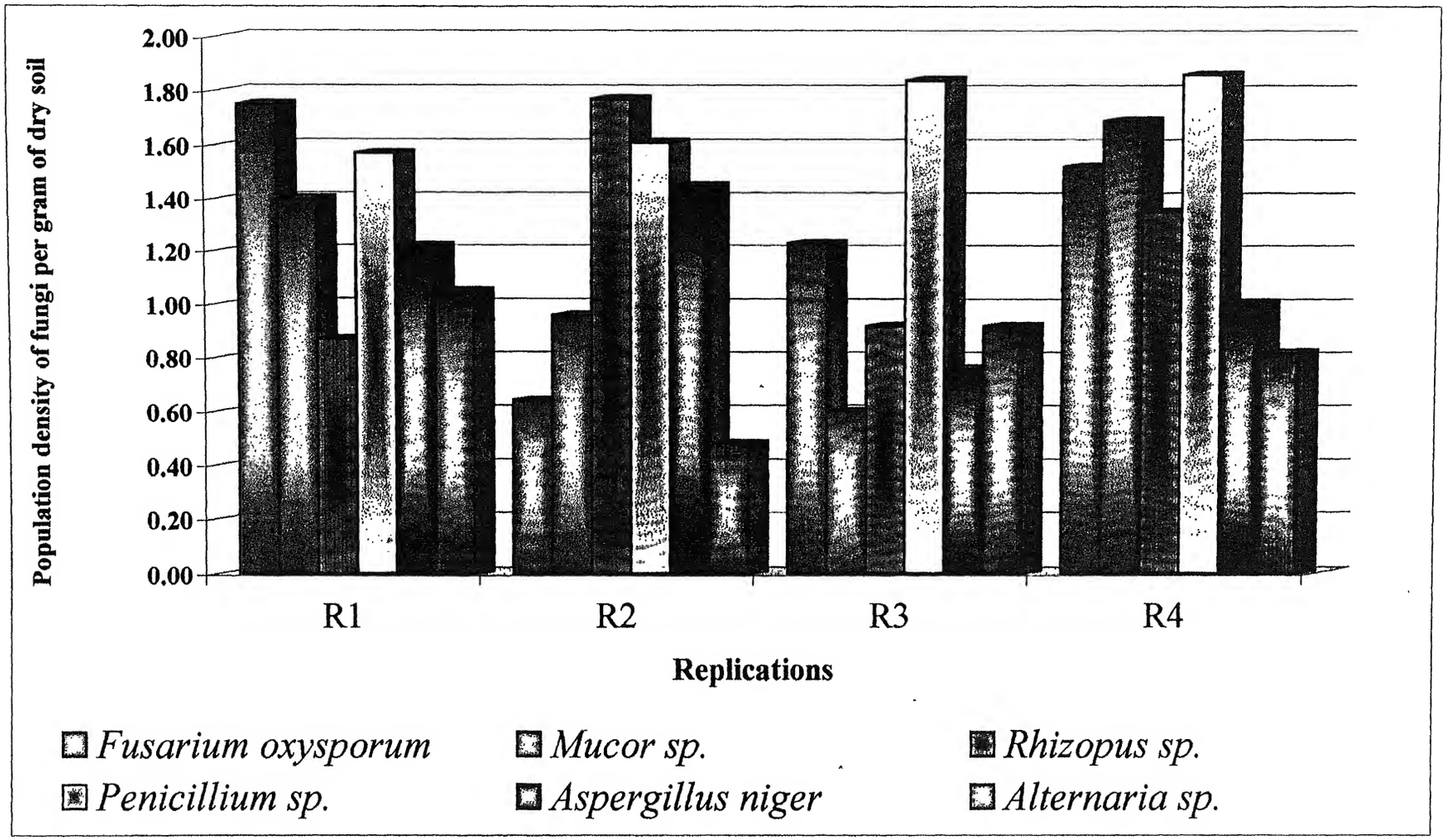


Table 33 : Population density of various rhizosphere fungi per gram of dry soil (10^3) at 30 DAS

Organisms	Treatments											
	T_0	%	T_1	%	T_2	%	T_3	%	T_4	%	T_5	%
Fusarium oxysporum	1.4	20.23	1.11	20.48	1.00	20.44	1.46	20.33	1.12	25.00	0.86	21.88
Mucor sp.	0.92	13.29	0.71	13.22	0.65	13.29	0.95	13.23	0.73	19.29	0.78	19.83
Rhizopus sp.	0.65	9.39	0.50	9.31	0.46	9.40	0.67	9.33	0.51	11.38	--	--
Penicillium sp.	1.5	21.67	1.16	21.60	1.05	21.44	1.54	21.44	1.18	29.33	1.09	27.73
Aspergillus niger	1.19	17.19	0.92	17.19	0.84	17.17	1.23	17.13	0.94	20.98	1.01	25.69
Alternaria sp.	1.26	18.20	0.98	18.20	0.89	18.20	1.30	18.10	--	--	0.19	4.83
Total	6.92	100	5.38	100	4.89	100	7.18	100	4.48	100	3.93	100

Variance (F-value) at 5%, due to replications - Significant (SEd ± 0.118 CD 0.243)
due to treatments - Significant (SEd ± 0.118 CD 0.243)

Population density of various Rhizosphere fungi per gram of dry soil (10^3) at 30 DAS

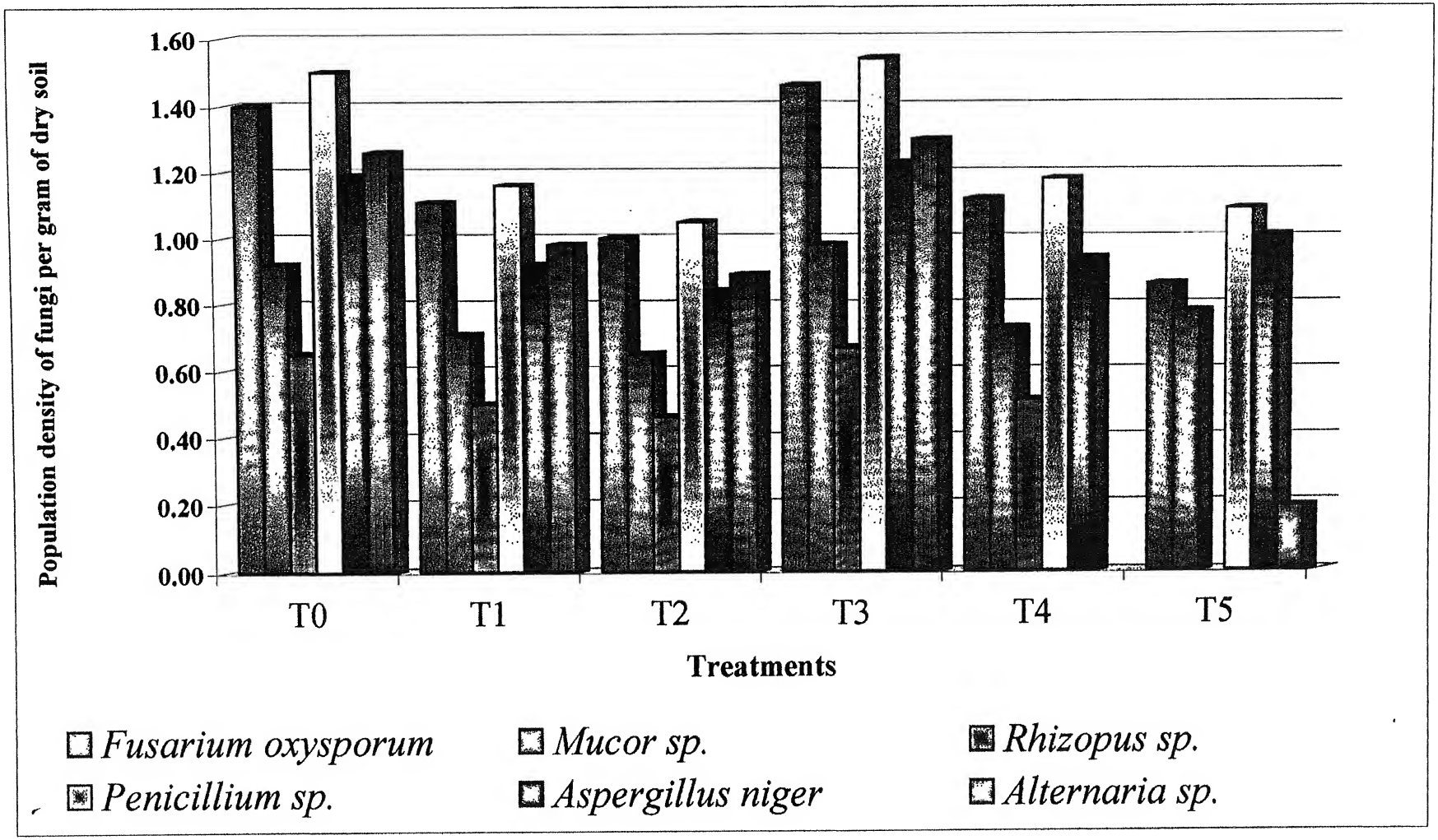


Table 34 : Population density of various rhizosphere fungi per gram of dry soil (10^3) at 60 DAS

Organisms	Treatments											
	T_0	%	T_1	%	T_2	%	T_3	%	T_4	%	T_5	%
Fusarium oxysporum	0.90	20.45	0.69	12.56	0.74	15.16	0.71	16.58	1.00	17.24	0.85	23.23
Mucor sp.	0.83	18.86	0.80	14.54	0.68	13.93	0.91	21.26	0.92	15.86	0.97	26.79
Rhizopus sp.	0.76	17.27	--	--	0.97	19.78	0.82	19.15	0.84	14.48	0.79	21.81
Penicillium sp.	0.93	21.13	1.29	23.49	0.77	15.77	--	--	1.03	17.75	--	1.09
Aspergillus niger	0.94	22.04	1.25	22.74	1.02	20.09	1.07	25.00	1.07	18.44	1.01	27.90
Alternaria sp.	--	--	1.46	26.59	0.68	13.93	0.75	17.52	0.92	15.86	--	--
Total	4.36	100	5.49	100	4.86	100	4.26	100	5.78	100	3.62	100

Variance (F-value) at 5%, due to replications - Non-significant
due to treatments - Non-significant

Population density of various Rhizosphere fungi per gram of dry soil (10^3) at 60 DAS

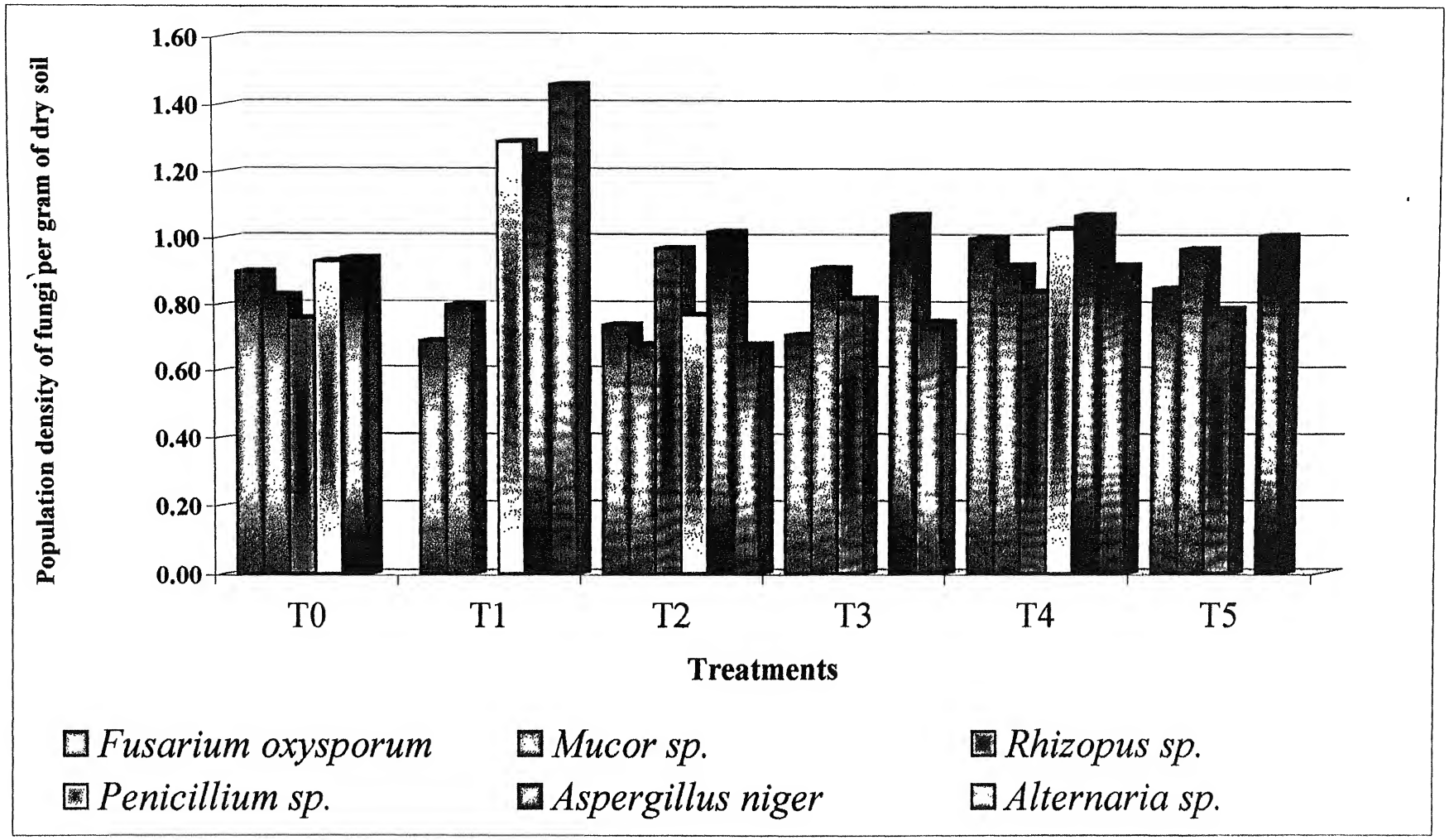


Table 35 : Population density of various rhizosphere fungi per gram of dry soil (10^3) at 90 DAS

Organisms	Treatments											
	T_0	%	T_1	%	T_2	%	T_3	%	T_4	%	T_5	%
Fusarium oxysporum	0.73	17.50	0.84	27.00	0.78	22.67	0.91	24.86	1.01	24.10	0.74	25.00
Mucor sp.	0.94	22.54	0.73	23.47	1.01	29.36	0.84	22.95	1.30	31.20	0.68	22.96
Rhizopus sp.	0.57	13.66	--	--	--	--	--	--	--	--	--	--
Penicillium sp.	0.96	23.02	0.67	21.54	0.61	17.73	0.77	21.03	0.79	18.85	0.63	21.28
Aspergillus niger	0.94	22.54	0.86	27.65	1.03	29.94	1.12	30.60	1.07	25.53	0.91	30.74
Alternaria sp.	--	--	--	--	--	--	--	--	--	--	--	
Total	4.14	100	3.10	100	3.43	100	3.64	100	4.17	100	2.96	100

Variance (F-value) at 5%, due to replications - Non-significant
due to treatments - Significant (SEd ± 0.82 CD 0.169)

Population density of various Rhizosphere fungi per gram of dry soil (10^3) at 90 DAS

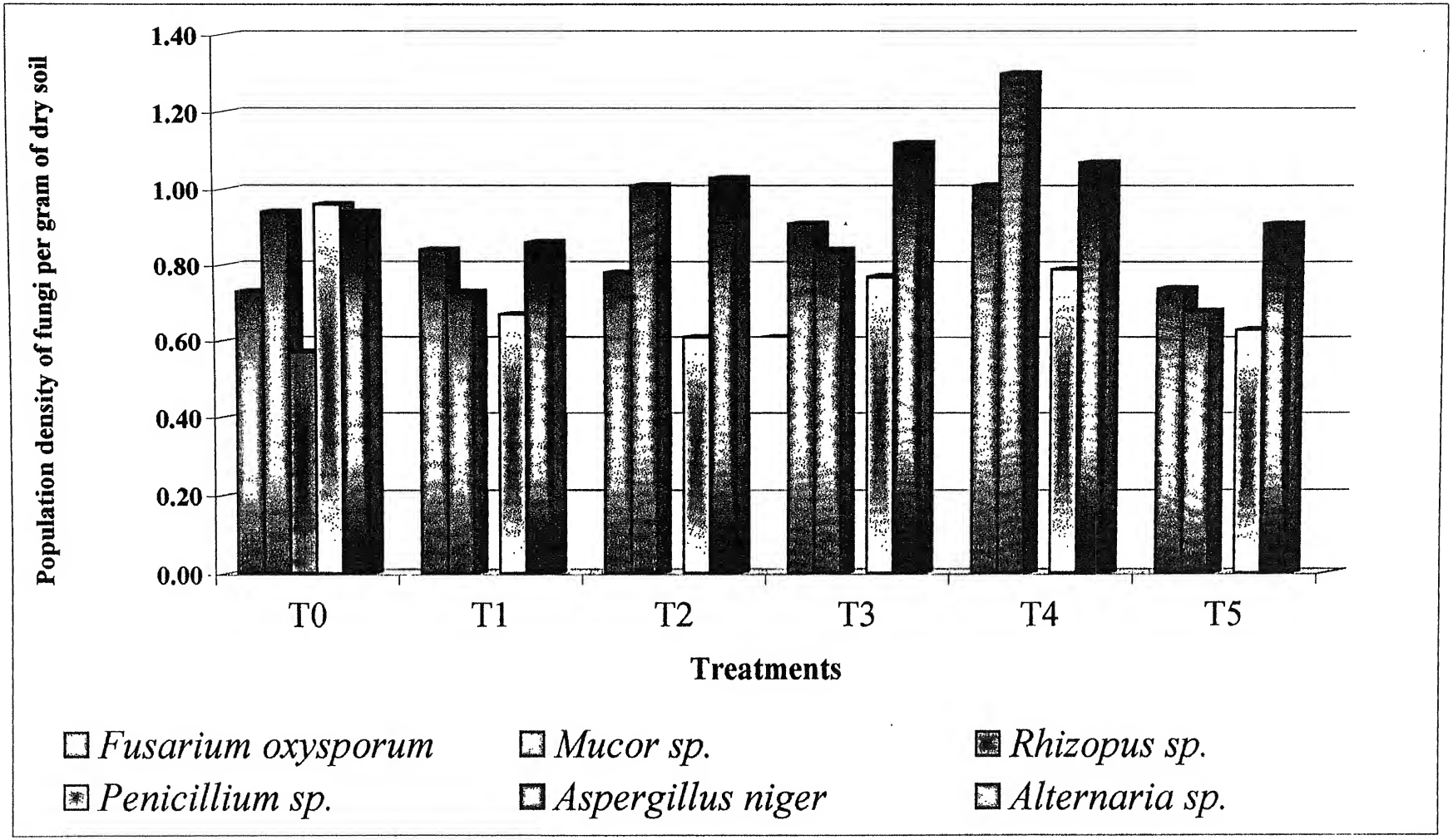
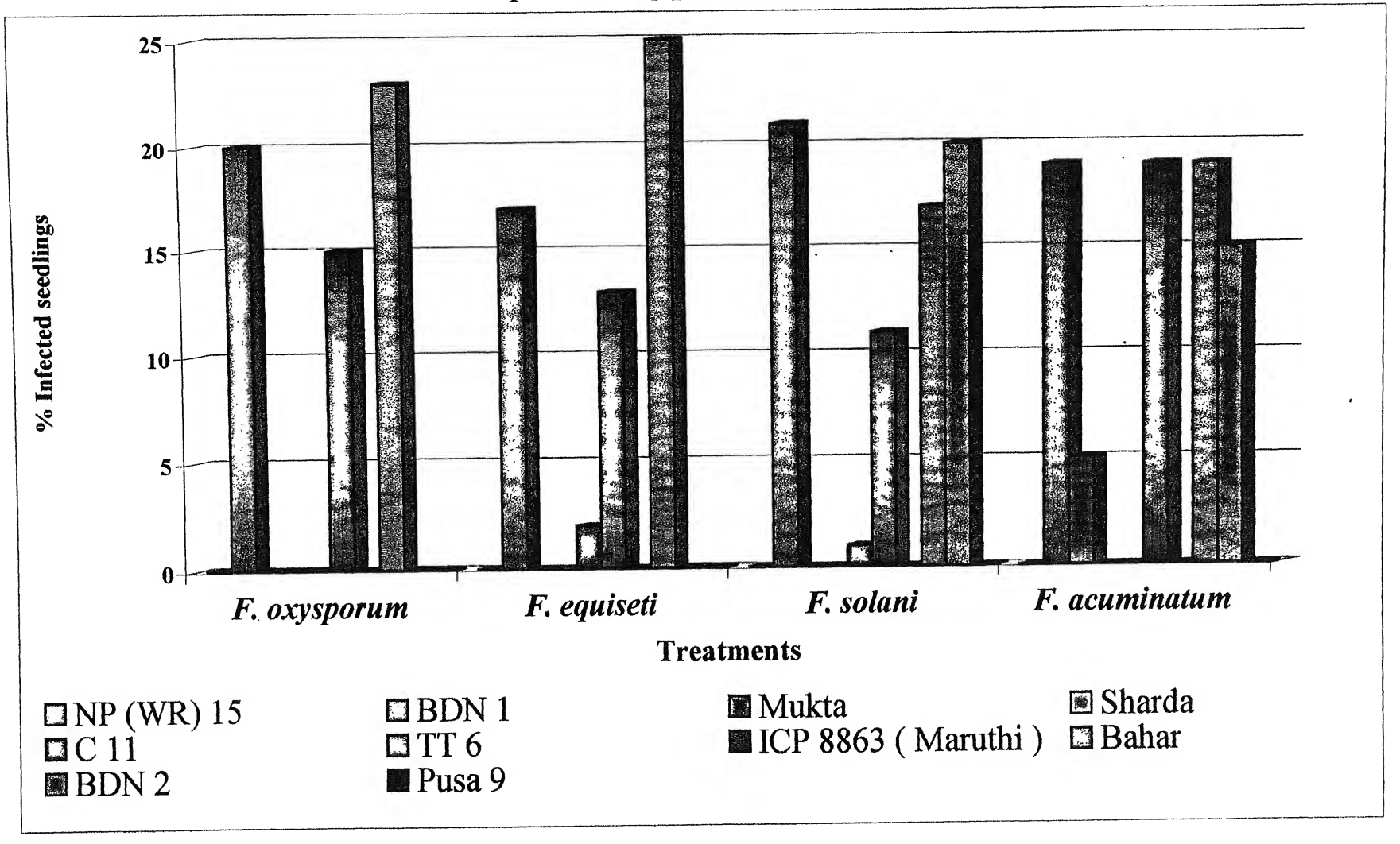


Table : 36

Varieties of Arhar crops showing percentage infection of *Fusarium* sp.

S.N0.	Varieties	% infected seedlings			
		F. oxysporum	*F. equiseti*	*F. solani*	*F. acuminatum*
1.	NP (WR) 15	00	00	00	00
2.	BDN1	20	17	21	19
3.	Mukta	00	00	00	05
4.	Sharda	00	00	00	00
5.	C11	00	02	01	00
6.	TT6	15	13	11	19
7.	ICP8863 (Maruthi)	00	00	00	00
8.	Bahar	23	25	17	19
9.	BDN2	00	00	20	15
10.	Pusa 9	00	00	00	00

Varieties of Arhar crops showing percentage infection of *Fusarium* sp.



Chapter 6
General Discussion

Wilt of Arhar (*Cajanus cajan*)
Fusarium solani

GENERAL DISCUSSION

Pulses form an integral part of the vegetarian diet in the Indian sub-continent. Besides being a rich source of protein, pulses provide two to three times more protein composition makes up for the deficiency of essential aminoacids in cereals and millets. Pulses fix atmospheric nitrogen in their roots and thus improve soil fertility. The tap root system opens the soil into deeper strata and heavy leaf drop increases the soil organic matter.

The per capita availability of pulses has declined from 64 g/capita/day (1951-56) to less than 48 g/capita/day as against the FAO/WHO's recommendation of 89 g/capita/day. If we take into account the total protein nutrition from other protein sources such as food grain, milk and its products, eggs, fish, meat etc. then 55 g/capita/day requirement of pulses may be the realistic target.

Pigeonpea is highly susceptible to plant disease. The diseases may be caused by fungi, bacteria and other micro-organism production losses of pigeonpea crops due to diseases are about 26% annually. In the present study isolation from the soils of the different fields of Allahabad and its adjacent areas carrying pigeonpea crop plants constantly yielded species of *Fusarium* besides a number of other fungi. The soil samples were collected monthly for one complete year. The data revealed much variation through the three years but no-significant difference was noted in number of colonies isolated from different areas in a particular

month. The soil of whole regions were found to be sandy loam (Gangetic alluvial) and loam. It was observed that soil of all regions contain maximum number of *Fusaria* during October and it was interesting to note that the soil conditions as well as climatic condition were optimum and comparatively better than the other months.

The rhizosphere mycoflora was different both qualitatively and quantitatively from the non-rhizosphere in all the seasons which agrees with the findings of Timonin (1940), Roy *et al.*, (1980) and Antique *et al.*, (1982). An increase in the number of rhizosphere fungi is perhaps due to "rhizosphere effect" (Hiltner, 1904; Rouatt and Katznelson, 1961; Antique *et al.* (1982). The fluctuation of fungal population in the non-rhizosphere and rhizosphere region may be due to edaphic factors viz. soil moisture, pH, temperature organic matter etc. (Alexander, 1977). Soil texture and pore space between soil particles also affect the growth of fungi and micro-organisms (Shaw, 1952).

Controversial views have been reported regarding the distribution of soil fungal flora in different seasons. According to Christensen, (1969), there is seasonal variation in the distribution of soil fungal flora but Gams and Domesh, (1969), have reported that seasons did not affect the quantitative as well as qualitative variations in the soil fungal flora. There is increase in the fungal population in rainy season due to relative humidity resulting in an increased in the moisture level of soil. In the present study similar pattern was found. The fungal

population increases in the rainy season and decrease in summer. It may be due to low moisture content and high temperature but it rain starts the moisture level of increases and fungi are encouraged for growth and sporulation.

The fungal population decrease with increase in the soil depths. The reduction in number of fungi with increasing soil depth may be due to the reduction in organic matter and oxygen, and increase carbon dioxide. *Pencillia, Trichoderma, Aspergilli* and *Fusaria* seems to tolerate the fluctuations in environmental conditions (Phanasenko, 1967). Hence it may be concluded that these antagonists have wide ecological spectrum. The present study agrees with the report of Upadhyay and Rai, (1979).

Fungi isolated from seeds of local varieties, fifty-eight species of twenty-three genera were present in which sixteen species of eight genera were internally seed-borne and remaining externally seed-borne.

The fungi were isolated from soil samples and culture maintained by incubator at 27°C ± 1°C. In soil samples 43 species of fourteen genera were found, in which eight species of *Fusarium* and remaining others. Twenty nine species were isolated from varietal seed samples in which only two species of *Fusarium* and others viz. *Curvularia* sp., *Alternaria* sp., *Aspergillus* sp., *Pencillium* sp., *Botrytis* sp., *Chaetomium* sp., *Drechslera* sp., *Rhizoctonia* sp., and *Helminthosporium* sp. was pathogenic. The nature of diseases observed were wilt,

seed-rot, root-rot, stem-rot, seedling blight, leaf spot. Apart from the above, four species of *Fusarium* and one species of *Alternaria* were found pathogenic causing wilt, seedling rot and leaf-spot in pigeonpea crops. Among the pathogenic *Fusaria, F. oxysporum, F. solani, F. equiseti, F. acuminatum* were observed most frequently to cause a number of diseases viz. seed and seedling rot, wilt, pod-rot and root-rot.

Soil borne fungal pathogens are influenced by soil water factor which is an important factor for better growth and survival of such fungi (Cook and Papendick, 1972). The propagates of saprophytic and pathogenic fungi survive in the root region of plants in adverse conditions by colonizing organic debris and producting chlamydospore and sclerotia (Garrett, 1967).

Seeds of almost all crops are invariably bound to carry on or with or within itself a large number of micro-organisms like fungi, actimonycetes, bacteria and viruses which collectively designated as 'seed-borne' mycoflora is used. The fungi invades the seeds either during its development before harvest or during transit after harvest and threshing to cause discoloration and shriveling of seeds (Machacek and Greuney, 1933 ; Kommedahl *et al.*, 1955).

Fourteen species of *Aspergillus* isolated, pigeonpea seeds were reported throughout the world (Husain and Ahmed, 1971; Shukla and Bhargava, 1977; Ellis *et al.*, 1978). Among them, *A. flavus, A. niger, A candidus, A. fumigatus, A.*

sydowii, *A. tamarrii* and *A. terreus* produced seed rot, pre and post emergence mortality of seedlings (Husain and Ahmed, 1971). *Aspergillus flavus* and *A. niger* produced seed and seedling-rot (Shukla and Bhargava, 1977).

Fusarium semitectum which was detected from internal tissues of seed-coat, cotyledons and occasionally from embryo (Ellis *et al.*, 1977), *F. oxysporum* was also detected from seeds and considered as a dominant fungus caused pod spot or seed-rot or seedling-rot and leaf-blight (Singh, 1988). Dwivedi and Tandon (1975) reported *F. udum*, a soil-borne wilt causing pathogen, to be seed borne fungus as they recorded a fair amount of infection of this pathogen in seeds.

Since the beginning of the study of the microbiology it has been recognized that soil is a great reservoir of micro-organisms. All types of micro-organisms live in the soil, the important ones being fungi, bacteria, actinomycetes, algae, protozoan and small animals. These organisms live in a constant state of competition and struggle for survival, as does the micro-vegetation on the surface of the earth. In this struggle some of the species become dominant and some remain-sub-dominant and a sort of dynamic equilibrium is the result of a set of conditions prevailing in a particular place and time. In the case of plant pathogens there is a wealth of information on such interaction (Baker and Synder 1965; Toussoun *et al.* 1970; Baker and Cook, 1974). It is worth while to quote Baker and Cook (1974) : "Man does not know the total rester of micro-organisms in a single

tiny lump of soil, let alone their complex interaction with each other and with their physical and chemical environment".

A number of *Fusaria* were found from the soils of various fields and studies on their morphological characters indicated that they were isolates of *Fusarium oxysporum, F. solani, F. equiseti* and *F. acuminatum*. Pathogenicity tests of these isolates revealed that all of the four species caused seedling rot and wilting of the above crop plants. The incidence of wilting in relation to different soil sand composition was studies and the highest percentage of wilting was observed in the pots containing soil only by all the pathogens.

In spite of the recent extensive studies by several investigators on physiology, pathology and biochemistry of the pathogenic fungi, the point of convergence regarding their general behaviour has not been reached so far. A sailent land mark of such studies has been the apparent diversity of responses manifested by organisms to the compounds encountered in nature. A comparative knowledge of the subject might be of some aid in under standing the relationship between the host and the parasite and then may be control or minimise the diseases. However, efforts have been, made to correlate the results obtained in various experiments and certain conclusions have been drawn where possible.

Garber (1956) emphasized the need for an adequate nutritional environment in the host to permit the potential proliferation and metabolism of the infective

parasite. Detailed physiological investigations were carried out on these four species of *Fusarium* to observe the effect of different media, pH, temperature and vitamin.

In present study nine different media were tried, modified Asthana and Hawker's mediam 'A' provided fair growth and excellent sporulation of all the four species of *Fusarium*. The medium was single in constituation and was possible to modify the various ingredients in it and hence, it was selected as a basal medium for the physiological studies of the present organisms. Among Richard's medium gave maximum dry wt. and excellent sporulation, no chlamydospore formation, in all four species of *Fusarium*.

Agarwal 1958 had grown *Curvularia pennisati* on a number of media and had found that Asthana and Hawker's medium 'A' was quite suitable for this organism. The behaviour of *F. oxysporum, F. vasinfectum* which causes the cotton wilt in the USA and India was studied by Mundkur (1936), observed that the American strain was favoured under acidic condition, while the Indian strain developed best in alkaline reaction. Growth and reproduction of fungi are profoundly influenced by hydrogen-ion concentration of the substrate upon which they grow. The study indicated maximum dry wt. and excellent sporulation of all the four species of *Fusarium* except *F. equiseti* at pH 5.5 No chlamydospores were this pH. It was found that in all those cases where the original pH 7.5 to 7.8. It was found that in all those cases where the original pH was low the final pH increased

but in the cases where it was high, the pH decreased. Lowering of pH in case of media with higher initial pH was possible due to absorption of carbon dioxide produced by the fungus in the process of respiration.

Ross, (1960) reported that *Fusarium culmorum, F. sambucinum* var. *coeruleum* and *F. oxysporum* grow well at pH 5 to 8. Bhargava (1962) found that optimum range for growth was 4.5 to 6.5 for *Fusarium solani, Botryodiploidia ananassae* and *Macrophomina phaseolina.*

Temperature is an important environmental factor affecting the metabolic activities of the fungi. It is evident from the study that growth, sporulation and chlamydospore formation of the present organisms were prominently influenced by temperatures variation. All the four species of *Fusarium* could grow between a range of 10°C to 35°C. Their good growth and excellent sporulation were recorded at 25°C. No chlamydospore were formed at 25°C except in case of *F. solani* and *F. equiseti.* Chlamydospore formation were better in all the four species of *Fusarium* when the temperature was below and above 25°C.

Ross, (1960) reported that *Fusarium culmorum, F. sambucinum var, coeruleum* and *F. oxysporum* grow best at 28°C. Bhargava, (1962) obtained best growth and sporulation of *F. solani* at 25°C. Bhatnagar, (1967) observed a considerable variation in the production of microspores and macrospores in two isolates of *F. solani* and reported that as the temperature increased the production

of chlamydospores increased and at 34°C only the later were formed. According to Joffe and Palti, (1972), in cultures, isolates of *F. solani* grow best at 24°C-32°C.

The response of *Fusarium* species towards vitamins has showed that the organism are partially heterotrophic as addition of a mixture of vitamins stimulated their growth. They did not require an external supply of ascorbic acid and nicotinic acid as these two vitamins have slightly suppressed their growth. *F. solani* also behaved similarly but it differe from the above three species, as it did not require an external supply of thiamins for the growth. Addition of this vitamin, however reduced its growth. This fungus on the other hand required some amount of biotin and nicotinic acid.

Similar results were obtained, Bhargava and Tandon (1963) for *Fusarium solani, Botryodiploidia ananassae, Macrophomina phaseolina* and Nolan (1970) for *Catanaria angullulae.*

Studies on Pathogenicity tests of four species of *Fusarium* indicated that *F. oxysporum* was most pathogenic as it could cause high percentage of wilting in the 'Arhar' seedlings.

Soil is a resting place for a wilt pathogen and it is a primary source of inoculum for on set of plant diseases. Our knowledge regarding factors affecting survival of various wilt and root-rot causing organism is however, very meagre. A number of soil factors like temperature, soil moisture or by amending the soil with

chemicals will help in controlling many diseases. The population of *fusaria* was considerably reduced at low or high temperature and high soil moisture. As it has been suggested by Norton, (1953), Dhingra and Sinclair, (1975).

Investigations on the extra-cellular production of toxin *in vitro* revealed that *F. oxysporum, F. solani, F. acuminatum* and *F. equiseti* were more efficacious in producing the toxins. It was interesting to note that they produced the high amount of fusaric acid *in vitro*. Thus the severity of infection caused by them might be correlated with its capacity to produce high amount of Fusaric acid.

In the present study fungi were isolated from the soils both cultivated and non-cultivated fields and some of them well known as wilt causing pathogen. Isolation from different fields of Allahabad district and it's adjacent regions carrying pigeonpea crops constantly yielded species of *Fusarium* besides a number of other fungi.

In leguminaceae family the seeds after harvest became infected through pods and the infection involved the seed-coat and usually considerable parts of the cotyledon. The site of infection was the fleshy cotyledons, which provided a rich nutritious base for the seedlings as well as for the pathogens and possibly accounted for the important role of seed transmission of these pathogens.

Alternaria and *Fusarium* species provided example of serious seed-borne fungi that penetrated from the vascular system through the funiculus into the seed.

Rudolph and Harrison, (1945), isolated *F. moniliforme, F. oxysporum* and *F. equiseti* from vascular bundles in all parts of cotton including the internal tissue of the seed. According to Klisiewiez, (1963), *Fusarium oxysporum, Fusarium* sp. *carthami* spreaded intra and intercellularly in the tissue of the pericarp and seed-coat of safflower. *Botrytis cinerea* had been proved to be seed transmitted in *Viciafaba* and *V. villosa,* Noble and Richardson, (1968) and controlled effectively by hot water treatment Bikmukhametova, (1963).

In the present study, twelve species of fungal pathogens were isolated from internal tissue of the seeds of two cultivars viz. UPAS-120 and Pant A-8508. In which *Alternaria alternata* species was found higher percent seed infection in both cultivars. Discoloured and shrivelled seed were high percentage incidence by *Alternaria alternata* than other species in cultivar UPAS-120.

Alternaria species were reported as an important group of fungal species associated with pigeonpea seeds (Husain and Ahmed, 1971; Ellis *et al.*, 1977). Among them, *A. alternata* produced pre and post emergence mortality due to seed rot, seedling blight and leaf spot on adults plants (Husain and Ahmed, 1971; Lokesh *et al.*, 1987).

For better management and to promote vigorous crop growth which make the plants to escape infection or to survive the infection, the rule 'Prevention is better than cure' should be adopted. Here the term 'Prevention' confers the use of

physical chemical and biological agents as seed treatment for protecting the seed, seedlings and plants against the infection by seed and soil-borne pathogens.

The American Phytopathology Society, (1943), defined a fungicide as a chemical or physical agents that kills, or inhibits, the development of fungus spores or mycelium. Horsfall *et al.*, (1940), where among the first to suggest that the protective value of fungicides in the field could be predicted on the basis of laboratory bioassay tests. Horsfall considered that fungi toxicity and tenacity were the two fundamental components of potential protective value against plant diseases in the field.

One purpose of chemical seed treatment was to inactivate surface borne fungi that cause seed decay or seedling blights. Chemical seed treatment was used to create a protective zone around the seed through which soil borne micro-organisms failed to penetrate. Appropriate treatment of seeds got rid of the seed-borne pathogens and controlled to a large extent diseases that would have results otherwise.

Most protective fungicides had a relatively short effective residual life and consequently were requires to be reapplied to maintain residual protection of susceptible tissues. This practice was further influenced by the frequency of rainfall and such most fungicides were renewed at 10-14 days. Seed-borne

infection of *Fusarium semitectum* in soybean was controlled by seed treatment, Saharan and Gupta, (1974).

Eleven fungicides used in the present study for seed treatment. It was found that Bavistin-50 WP, Bavistin 25 SD + Thiram, Bavistin 25 SD and Captan, eliminate the entire mycoflora associated with pigeonpea seeds followed by vitavax in which all the fungal species were eliminated except few colonies of *Fusarium*.

In the present study for controlling the disease by seed treatment with biological agents by two antagonistics viz. *Chaetomium globosum* and *Trichoderma viride* against fourteen species of different fungal pathogen. In which were *T. viride* treated seeds eliminated entire pathogenic flora except *Aspergillus flavus, A. niger, Botrytis cinerea, Fusarium moniliforme* and *F. semitectum*, whereas *Chaetomium globosum* treated seeds reduced the incidence of pathogens it did not eliminate the infection of pathogens except *Cladosporium cladosporoides* which was completely checked, However both the antagonists were found to reduce the incidence of each and every pathogens.

According to Harman *et al.*, (1981); *Trichoderma hamatum* controlled seed rot of pea if soil temperature was 17-34°C and seed had been treated with a suspension of conidia of more than 16^6 per ml. Addition of chitin to seed coat increased the soil population density of *T. hamatum*.

Hubbard *et al.*, (1983); reported that *T. hamatum* applied as conidia to peas colonizes and coats and protect them from seed rot caused by *Pythium* sp. Sivan *et al.*, (1984); reported that pea seeds treated with conidia of *T. hamatum* were better protected from *Pythium aphanidermatum* than coated with *T. harzianum*.

Experiment dealing with effect of fungicides in soil on four species of *Fusarium* population, indicate that the effect of Topsin m and Topsin at both concentration (100 and 500 ppm) were show inhibitory growth of all *Fusarium sp.* Eight fungicides used, out of Benlate at higher concentration showed inhibitory growth of all species of *Fusarium* and other was less effective at both concentration.

Leaf extract of some medicinal plants which were found effective during spore germination were also tried for controlling the wilt of pigeonpea crops at seedling stage. Neem (*Azadirachta indica*), at 100% concentration, completely checked the spore germination of all the four *Fusarium* sp. and others, were some effective to check the spore germination of all *Fusarium* sp..

Leaf extract of *Bougainvillea glabra* and *Piper betle* were inhibitory to *Pythium monosperum* Alice, (1984), partial inhibition of *Helminthosporium sativum* by leaf extract of *Lowsonia inermis* was reported by Tripathi *et al.*, (1978). Maximum inhibition of *Drechslera oryzae* was obtained with pippermint leaves followed by *Piper nigrum* seed and garlic extract (Alice and Rao, 1987).

Crude leaf extracts of *Amaranthus spinosus, Legenaria siceriaria, Nerium indicum* and *Solanum nigrum* were completely inhibitory to uredospore germination of *Puccinia, helianthi*, Wadhwani *et al.*, 1986).

The effect of different neem products on rhizosphere mycoflora was observed in which Neem shield give more effective results than other neem products at all stages of plant growth and fungal population reduced too.

Plant breeders and plant pathologists in the past concentrated primarily to evolve varieties with high degree of resistance against specific pathogens or few races and biotypes of pathogens characterised by specific host parasite interaction and this type is designated as 'Vertical resistance'. In contrast to vertical resistance, another type is met which is fairly non specific with moderate level of resistance against a large number of pathogens or races, which is commonly known as 'field or horizontal resistance'.

In breeding for disease resistance, the aim should be to overlap such variety that may have characters which may be mechanical or non specific chemical substances as inhibitors or can induce enzymatic reaction resulting in inhibition of pathogens or may induce specific host parasite reaction. Numerous examples of successful breeding of plant for plant disease-resistance were cited by Caldwell, (1966).

In present study out of ten varieties were tested against four species of *Fusarium*. In which NP (WR) 15, Sharda, Pusa 9 and ICP 8863 (Maruthi) were found complete resistance to all *Fusarium* sp.

The application of leaf extract, Neem products and resistant varieties in controlling the plant diseases could be a good alternative to chemical fungicides and pesticides. These products are biodegradable therefore do not alter the soil physio-chemical characteristics.

Chapter 7
Summary

Wilt of Arhar (*Cajanus cajan*)
Fusarium oxysporum

SUMMARY

An extensive survey of various fields and markets, opened seed agencies of survival places situated in and around Allahabad region viz. Agriculture Farm Allahabad University, Chaka Block, Bara Region, Karchana Region, Koraon Region, Meja Region, Soraon Region, Begum Sarai, Khushroo Bagh and Chandra Shekhar Azad Park were made. Warcup and Serial Dilution Agar Plate technique were used for detection of soil-borne and Standard Blotter Method for seed-borne fungi.

Twenty varietal samples of seeds were collected from, N.D. University of Agricultural & Technology. Kumarganj, Faizabad (U.P.), Pulse Research Directorate, Kanpur, Division of Mycology and Plant Pathology, IARI, New Delhi, Legume Section C.S.A. University of Agricultural & Technology, Kanpur and U.P. Seeds and Tarai Development Corporation Regional Station, Kalyanpur, Kanpur.

Fungi were isolated purified and maintained on Potato Dextrose Agar, Malt-extract Agar and Peptone Dextrose-Rose Bengal Agar Media were used. Morphological characters were carried out and identification were observed.

Fungi isolated from local varieties of seeds were found fifty eight species of twenty three genera in which sixteen species of eight genera were found internally seed-borne and remaining externally seed-borne.

In the observation twenty nine species were isolated from varietal seed samples and fourty three species from soil and infected plants. In which two of Mastigomycotina, four of Zygomycotina, three of Ascomycotina and five of Deuteromycotina were found. Details are given below :

Fungi isolated from seeds and soils of various Arhar crop fields of Allahabad District

Regions	Soils	Seeds
Agriculture Farm University of Allahabad		
	Fusarium oxysporum	**Fusarium semitectum*
	F. solani	**Fusarium moniliforme*
	F. equiseti	**Curvularia lunata*
	F. moniliforme	*Microphomina phaseolina*
	Aspergillus flavus	**Alternaria alternata*
	A. niger	**A. longissima*
	Rhizopus stolonifer	**Aspergillus flavus*
	R. sp.	*A. niger*
	Penicillium notatum	*Botrytis cinerea*
	Saprolegnia sp.	*Cephalosporium sp.*
	Mucor mucedo	*Chaetomium globosum*
	M. javanicus	*C. olivaceum*
Chaka Block	*Mucor hiemalis*	*C. setosum*
	M. mucedo	*C. offine*

	M. spinosus	**Cladosporium cladosporiodes*
	M. racemosus	**Colletotrichum dematium*
	Fusarium solani	**Curvularia lunata*
	F. acuminatum	*Drechslera tetramera*
	F. equiseti	*Helminthosporium tetramera*
	F. oxysporum	*Bipolaris tetramera*
	Aspergillus niger	**Fusarium moniliforme*
	A. flavus	**F. semitectum*
	A. nidulance	*Mucor mucedo*
	A. fumigatus	*Mucor sp.*
Bara Region	*Penicillium sp.*	*Pencillium oxalicum*
	Mucor javanicus	*Phoma sp.*
	M. circinelloides	**Phyllosticta cajani*
	M. ramosissimus	**Rhizoctonia bataticola*
	M. spinosus	**Rhizoctonia solani*
	Rhizomucor miehei	*Rhizopus arrhizus*
	Rhizomucor pasillus	*Rhizomucor pasillus*
	Fusarium oxysporum	*R. nigricans*
	Fusarium equiseti	**Trichothecium roseum*
	F. solani	*Phoma emblica*
	Rhizopus equinus	*Alternaria sp.*
	R. cohnii	*Fusarium equiseti*
	R. microsporus	**Aspergillus flavus*
	Aspergillus niger	*A. sogae*
	A. nidulance	*A. wentii*

Karchana Region	*Pilobolus klunii*	*Pencillium notatum*
	Rhizopus arrhizus	*Phytophthora infestans*
	R. cohnii	**Fusarium oxysporum*
	R. microsporus	*Aspergillus nidulance*
	Mucor mucedo	*A. fumigatus*
	M. hiemalis	*Rhizopus arrhizus*
	M. spinosus	*R. microsporus*
	Fusarium semitectum	*Rhizomucor miehei*
	F. equiseti	*R. pasillus*
	F. moniliforme	*Phyllosticta cajani*
	F. roseum	*Rhizoctonia bataticola*
	Aspergillus flavus	*Assochyta phaseolorum*
	Aspergillus niger	*Colletotrichum lindemuthianum*
	Pencillium sp.	**C. dematium*
Koraon Region	*Alternaria alternata*	*Aspergillus niger*
	Phoma emblica	*A. fumigatus*
	Fusarium solani	*Rhizopus eqiunus*
	F. decentcelluelare	*R. cohnii*
	F. oxysporum	*Aspergillus nidulance*
	Aspergillus oryzae	**A. flavus*
	A. sogae	**Fusarium semitectum*
	A. wentii	**F. acuminatum*
	Pencillium notatum	*Drechslera cajani*
	P. sp.	*Botrytis cinerea*
	Mucor mucedo	*Cephalosporium sp.*

Meja Region	*M. javanicus*	*Mucor mucedo*
	Fusarium oxysporum	*M. javanicus*
	F. equiseti	*M. ramosissimus*
	F. acuminatum	**Cladosporium cladosporiodes*
	Alternaria alternata	**Alternaria longissima*
	Alternaria oryzae	**A. alternata*
	Aspergillus niger	*Chaetomium globosum*
	A. phailiseptus	*C. setosum*
	A. clavatus	*Helminthosporium tetramera*
	Pencillium sp.	*Phoma sp.*
	Mucor spinosus	**Rhizoctonia bataticola*
	M. ramosissimus	*Fusarium equiseti*
	M. hiemalis	*Rhizopus stolonifer*
Handia Region	*Fusarium oxysporum*	*R. arrhizus*
	F. semitectum	*R. nigricans*
	F. solani	*Rhizomucor passilus*
	F. equiseti	**Rhizoctonia solani*
	Mucor mucedo	**Alternaria alternata*
	M. spinosus	*Aspergillus nidulance*
	Rhizopus stolonifer	**A. flavus*
	R. arrhizus	*Macrophomina phaseolina*
	Rhizomucor miehei	*Rhizoctonia solani*
Phoolpur Region	*Macrophomina phaseolina*	*Rhizopus microsporus*
	Curvularia lunata	*Botrytis cinerea*
	Aspergillus flavus	**Trichothecium roseum*

	A. oryzae	*Phoma emblica*
	A. fumigatus	*Aspergillus flavus*
	A. spinulosum	*A. wentii*
	Rhizopus stolonifer	*Ascochyla phaseolorum*
	R. arrhizus	**Colletotrichum dematum*
	Rhizomucor miehei	*Bipolaris tetramera*
	F. oxysporum	*Cephalosporium sp.*
	F. solani	**Alternaria longissima*
	F. equiseti	*Mucor mucedo*
	F. semitectum	*M. javanicus*
	Mucor spinosus	*M. ramosissimus*
	M. ramosissimus	*Rhizomucor miehei*
Soraon Region	*Fusarium oxysporum*	*Cladosporium oxysporum*
	F. solani	**Fusarium decentcelluelare*
	F. equiseti	**Fusarium roseum*
	F. acuminatum	**F. semitectum*
	Phytophthora infestans	*Colletotrichum lindemuthianum*
	Penicillium notatum	**Cladosporium cladosporiodes*
	P. olavigerum	*Mucor hiemalis*
	P. sp.	*M. racemosus*
	Talaromyces vermiculotus	*M. mucedo*
	Rhizopus equinus	*Phyllosticta cajani*
	R. arrhizus	*Drechslera tetramera*
	Rhizopus microsporus	**Fusarium oxysporum*

	Aspergillus flavus	**F. acuminatum*
	Aspergillus niger	*Rhizopus nigricans*
	Mucor mucedo	*R. arrhizus*
	M. racimosus	*Rhizomucor pasillus*
	Alternaria alternata	*Phoma sp.*
Begum Sarai	*Fusarium solani*	**Curvularia lunata*
	F. oxysporum	*Ascochyta phaseolorum*
	F. moniliforme	*Macrophomina phaseolina*
	F. semitectum	*Aspergillus niger*
	Mucor spinosus	*A. nidulance*
	M. ramosissimus	*A. fumigatus*
	M. mucedo	*Penicillium notatum*
	Aspergillus flavus	**Fusarium moniliforme*
	A. niger	**Trichothecium roseum*
	A. phailiseptus	**Colletotrichum dematum*
	A. clavatus	*Chaetomium globosum*
	Rhizopus stolonifer	*C. affine*
	R. arrhizus	*Cephalosporium sp.*
	Rhizomucor miehei	**Alternaria alternata*
Chandra Shekhar Azad Park	*Alternaria alternata*	**A. longissima*
	F. solani	*Cladosporium oxysporum*
	F. semitectum	*Macrophomina phaseolina*
	Aspergillus flavus	**Rhizoctonia solani*
	A. niger	**Trichothecium roseum*

	A. nidulance	*Mucor spinosus*
	A. fumigatus	*M. mucedo*
	Mucor spinosus	*Aspergillus clavatus*
	M. mucedo	*A. phailiseptus*
	M. racimosus	*Pencillium sp.*
	Rhizopus stolonifer	*P. notatum*
	R. arrhizus	**F. oxysporum*
	R. sp.	**F. semitectum*
	Rhizomucor miehei	*F. equiseti*
Khushroo Bagh	*Aspergillus flavus*	**F. moniliforme*
	A. nidulance	*Rhizopus stolonifer*
	A. fumigatus	*R. arrhizus*
	A. phailiseptus	*Botrytis cenaria*
	A. clavatus	*Phytophthora infestans*
	Fusarium oxysporum	*Phoma sp.*
	Fusarium solani	**Rhizoctonia bataticola*
	F. semitectum	*Phyllosticta cajani*
	F. acuminatum	*Macrophomina phaseolina*
	F. equiseti	**Cladosporium cladosporiodes*
	F. moniliforme	*Cladosporium oxysporum*
	Mucor mecedo	**Trichothecium roseum*
	M. spinosus	**Fusarium decentcelluelare*
	M. hiemalis	**F. roseum*
	Rhizopus stolonifer	*Mucor mucedo*
	R. arrhizus	*M. hiemalis*

Rhizomucor miehei	*M. racemosses*
Alternaria alternata	**Fusarium moniliforme*
Alternaria oryzae	**F. semitectum*
Phoma emblica	**F. acuminatum*
Penicillium sp.	*F. equiseti*
	F. solani
	Ascochyta phaseolorum
	**Curvularia lunata*
	**Alternaria longissima*

* Denotes internally seed-borne

A number of *Fusaria* were found from the soil of various fields and studies on their morphological characters indicated that they were isolates of *Fusarium oxysporum, F. solani, F. equiseti* and *F. acuminatum*. Pathogenicity tests of these isolates revealed that all of the four species caused seedling rot and wilting of the above crop plants. The incidence of wilting in relation to different soil-sand composition was studies and the highest percentage of wilting was observed in the pots containing soil only, by all the pathogens.

In present study nine different media were tried, Modified Asthana and Hawker's mediam 'A' provided fair growth and excellent sporulation of all the four species of *Fusarium*. The medium was single in constituation and was possible to modify the various ingredients in it and hence, it was selected as a basal medium

for the physiological studies of the present organisms. Among Richard's medium gave maximum dry wt. and excellent sporulation, no chlamydospore formation, in all four species of *Fusarium.*

Growth and reproduction of fungi are profoundly influenced by hydrogen-ion concentration of the substrate upon which they grow. The study indicated maximum dry wt. and excellent sporulation of all the four species of *Fusarium,* except *F. equeseti* at pH 5.5 no chlamydospores were this pH. It was found that in all those cases where the original pH 7.5 to 7.8. It was found that in all those cases where the original pH was low the final pH increased but in these cases where it was high, the pH decreased. Lowering of pH in case media with higher initial pH was possibly due to absorption of carbon dioxide produced by the fungus in the process of respiration.

Temperature is an important environmental factor affecting the metabolic activities of the fungi. It is evident from the study that growth, sporulation and chlamydospore formation of the present organisms were prominently influenced by temperature variation. All the four species of *Fusarium* could grow between a range of 10°C to 35°C. Their good growth and excellent sporulation were recorded at 25°C. No chlamydospore were formed at 25°C except in case of *F. solani* and *F. equiseti.* Clamydospore formation were better in all the four species of *Fusarium* when the temperature was below and above 25°C.

The response of *Fusarium* species towards vitamins has showed that the organism are partially heterotrophic as addition of a mixture of vitamins stimulated their growth. They did not require an external supply of ascorbic acid and nicotinic acid as these two vitamins have slightly suppressed their growth. *F. solani* also behaved similarly but it differe from the above three species, as it did not require an external supply of thiamin for the growth. Addition of this vitamin, however reduced its growth. This fungus on the other hand required some amount of biotin and nicotinic acid.

Studies on Pathogenicity tests of four species *Fusarium* indicated that *F. oxysporum* was most pathogenic as it could cause high percentage of wilting in the 'Arhar' seedlings.

The fungi were isolated from soil samples and seed samples and culture maintained by incubator at 27°C ± 1°C. In soil samples fourty three species of different genera found, in which eight species of *Fusarium* and others. Fifty eight species were isolated from local variety of seeds and Twenty nine species were isolated from varietal seed samples in which only two species of *Fusarium* and others viz. *Curvularia sp., Alternaria sp., Aspergillus sp., Pencillium sp., Botrytis sp., Chaetomium sp., Drechslera sp., Rhizoctonia sp.,* and *Helminthosporium* sp. were pathogenic. The nature of diseases observed were wilt, seed-rot, root-rot, stem-rot, seedling blight, leaf spot. Apart from the above, four species of *Fusarium* and one species of *Alternaria* were found pathogenic causing wilt, seedling rot and

leaf-spot in pigeonpea crops. Among the pathogenic *fusaria, F. oxysporum, F. solani, F. equiseti, F. acuminatum* were observed most frequently to cause a number of diseases viz. seed and seedling rot, wilt, pod-rot and root-rot.

Investigations on the extra-cellular production of toxin *in vitro* revealed that *F. oxysporum, F. solani, F. acuminatum* and *F. equiseti* were more efficacious in producing the toxins. It was interesting to note that they produced the high amount of fusaric acid *in vitro*. Thus the severity of infection caused by them might be correlated with its capacity to produce high amount of Fusaric acid.

In the present study, twelve species of fungal pathogens were isolated from internal tissue of the seeds of two cultivars viz. UPAS-120 and Pant A-8508. In which *Alternaria alternata* species was found higher percent seed infection in both cultivars. Discoloured and shrivelled seed were high percentage incidence by *Alternaria alternata* than other species in cultivar UPAS-120.

Eleven fungicides used in the present study for seed treatment. These were found that Bavistin-50 WP, Bavistin 25 SD + Thiram, Bavistin 25 SD and Captan, eliminate the entire mycoflora associated with pigeonpea seeds followed by vitavax in which all the fungal species were eliminated except few colonies of *Fusarium.*

In the present study two biological agents antagonistics viz. *Chaetomium globosum* and *Trichoderma viride* were tested against fourteen species of different

fungal pathogens. In which *T. viride* treated seeds eliminated entire pathogenic flora except *Aspergillus flavus, A. niger, Botrytis cinerea, Fusarium moniliforme* and *F. semitectum*, whereas *Chaetomium globosum* treated seeds reduced the incidence of pathogens it did not the eliminate the infection of pathogens except *Cladosporium cladosporoides* which was completely checked, However both the antagonists were found to reduce the incidence of each and every pathogens.

Experiments dealing with effect of fungicides in soil on four species of *Fusarium* population, indicate that the effect of Topsin m and Topsin at both concentration (100 and 500 ppm) were show completely checked the growth of all *Fusarium* sp. Benlate at higher concentration showed inhibitory growth of all *Fusarium sp.* plantvax at higher concentration was some effective but others were failed in all concentration.

Leaf extract of some medicinal plants which were found effective during spore germination were also tried for controlling the wilt of pigeonpea crops at seedling stage. Neem (*Azadirachta indica*), at 100% concentration, completely checked the spore germination of all the four *Fusarium* sp. and others, except *Datura fastuosa* were some effective to check the spore germination of all *Fusarium* sp..

The effect of different neem products on rhizosphere mycoflora was observed in which Neem shield give more effective results than other neem products at all stages of plant growth and fungal population reduced too.

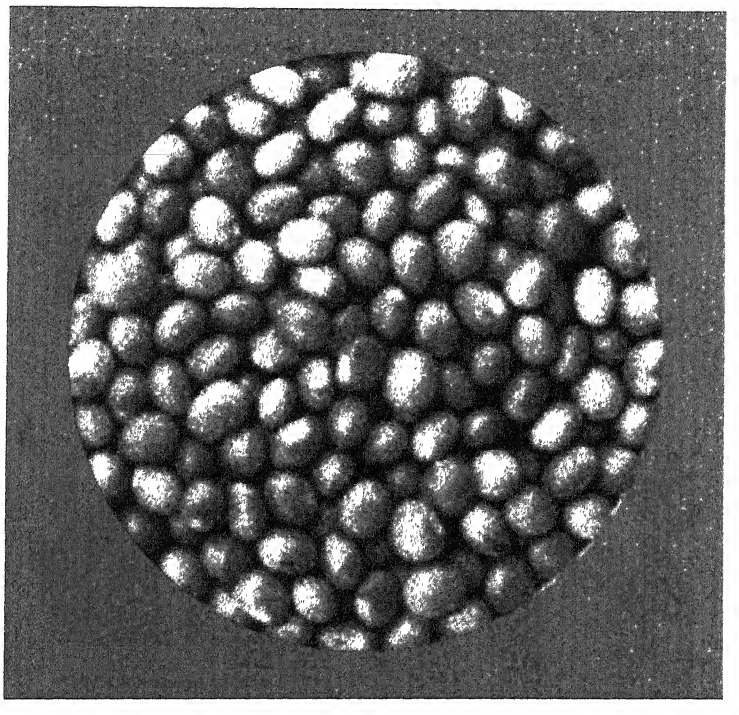
In present study out of ten varieties were tested against four species of *Fusarium*. In which NP (WR) 15, Sharda, Pusa 9 and ICP 8863 (Maruthi) were found complete resistance to all *Fusarium* sp.

In the present study it was found that fungi were present in soils both cultivated and non-cultivated fields and some of them were as wilt causing pathogen. In the present study isolation from different fields of Allahabad district and it's adjacent regions carrying pigeonpea crops constantly yielded species of *Fusarium* besides a number of other fungi.

The application of leaf extract, Neem products and resistant varieties in controlling the plant diseases could be a good alternative to chemical fungicides and pesticides. These products are biodegradable therefore do not alter the soil physio-chemical characteristics.

Chapter 8

Bibliography



Healthy Seeds of Arhar (*Cajanus cajan*)
Variety Bahar

BIBLIOGRAPHY

A.Q.A.C., 1970 : Official methods of analysis, Association of official analytic chemists, Washington, 11th Ed., pp. **16** : 214-215.

*Adametz, L., 1886 : Untersuchungen uber die modern pilze der Aekerkrume. Inagural dissertation, University of Leipzig.

Agarwal, G.P., 1955 : D. Phil. Thesis, University of Allahabad.

Agarwal, G.P., 1958 : *Phyton*, **11** : 143-151.

Agnihotri, V.P., 1963b : *Canad. Jour. Microbiol.*, **9** : 703-707.

Agnihotrudu, V., 1972 : *Proc. Ind. Acad. Bot.*, **75 B** : 54-64.

Agrios, G.N., 1988 : "Plant pathology", Acadmic Press Limited, London, **2**: 40-41.

Albritton, E.C. (Ed.), 1953 : *WADC Tech. Report.* 52-301. Wright Patterson Air Force Base, Chic.

Alexander, M., 1977 : Introduction to Soil Microbiology. 2nd ed. John Wiley, New York.

Alice D. and Rao, A.V. 1987 : Antifungal effect of plant extracts on *Drechslera orizae* (rice). *IRAN.* **12** : 28.

Alice, D., 1984 : Studies on antifungal properties of some plant extracts, M.Sc. Thesis, TNAU Coimbatore.

Ames, A., 1915 : *Phytopathology*, **6** : 11-19.

Anonymous, 1990-91 : Economic Survey Government of India.

Antique, M., A. Khan and D. Prakash, 1982 : *Indian Phytopath.*, **35** : 717-718.

Asthana, R.P. and L.E. Hawker, 1936 : *Ann. Bot.*, **50** : 325-344.

Backman, C.H., J.E. Kuntz and A.J. Riker, 1953 : *Phytopathology*, **43** : 441-447.

Baker, K.F. and R.J. Cook, 1974 : Biological control of plant pathogens, W.H. Freeman and Co., San Francisco, U.S.A..

Baker, K.F. and W.C. Snyder, 1965 : Ecology of soil-borne plant pathogens, Prelude to Biological control, Univ. of Calif. Press, Berkeley, Los Angeles and London.

Barnett, H.L., 1970 : *Mycologia*, **62** : 750-760.

Bhatnagar, G.C., 1967 : *Labdev J. Sci. Tech.* **5** : 68-69.

Bhargava, S.N. and R.N. Tandon, 1963 : *Pathologia et. Microbiol.*, **26** : 313-317.

Bhargava, S.N., 1962 : D. Phil. Thesis, University of Allahabad.

Biffen, R.H., 1905 : "Mendel's Laws of inheritance and wheat breeding," *J. Agric. Sci. Cumb.*, **1** : 4-48.

*Bikmukhametova, R.N., 1963 : *Trud. Bashkir : Sel-Shoz. Inst.* **11** : 19-22. (RAM 1965, 1936).

Bilgrami, K.S., 1956 : D. Phil. Thesis, University of Allahabad.

Bilgrami, K.S., 1964 : *Flora, Bd.* **154** : 81-88.

Bisht, V.S., Kannaiyan, J. and Nene, Y.L., 1988 : Methods of Metaloxyl application of control phytophthora blight of pigeonpea, International pigeonpea news letter, **8** : 9-11.

Bissett, J. and D. Parkinson, 1979 a : *Can. J. Bot.*, **57** : 1609-1629.

Bissett, J. and D. Parkinson, 1979b : *Can. J. Bot.* **57** : 1630-1641.

Bissett, J. and D. Parkinson, 1979c : *Can. J. Bot.* **57** : 1642-1659.

Booth, C., 1971 : The genus *Fusarium* C.M.I. Kew, Surrey England.

Bowen, G.D. and A.D. Rovira, 1976 : *Ann. Rev. Phytopathol.* **14** : 121-144.

Broadfoot, W.C. and M.W. Gormaok, 1941 : *Phytopathology*, **31** : 1058-1059.

Brooks, F.T. and C.G. Hansford, 1923 : *Trans. Brit. Mycol. Soc.*, **8** : 113-141.

Brown, G.E., 1974 : *Phytopathology.*, **64** : 539-542.

Burges, A., 1965 : In. Ecology of soil-borne plant pathogens, Ed. K.F. Baker and W.C. Synder, pp. 21-31. John Murray, London.

Caldwell, P.M., 1966 : *U.S. Dep. Agric. ARS Publ.* 33-110, Washington, D.C.

Campbell, M.E., 1938 : *Trans. Pey. Soc. Edinb.*, **30** : 411-436.

Cantino, E.C., 1948 : *Am. J. Bot.*, **35** : 238-242.

Chandra, S., Narang, M. and Srivastava, R.K., 1985 : Seed Science and Technology, **13 (3)** : 537-541., Dep. Bot., University of Allahabad, India.

Chandramohan, D. and A. Mahadevan, 1968 : *Experimentia,* **24** : 427.

Chaturvedi, C., 1961 : D. Phil. Thesis, University of Allahabad.

Chaturvedi, S.K. & Ali, M., 2002 :The Hindu Survey of Indian Agriculture,63-67.

Chaudhuri, H. and G.S. Sachar, 1934 : *Ann. Mycol.*, **32** : 90-100.

Chaudhuri, H. and M. Umar, 1939 : *Proc. Ind. Acad. Sci.*, **8 B** : 79-92.

Chaudhuri, H., 1939 : *Proc. Ind. Acad. Sci.*, **8 B** : 93-99.

Christensen, C.M., 1969 : *Phytopathology*, **59** : 1699-1702.

Christensen, J.J., 1963 : *Plant Disease Reptr.*, 56 : 173-175.

Clark, F.E., 1949 : Soil Micro-organisms and Roots, *Advan. Agron.*, 1 : 241-288.

Cochrane, V.W. and J.C. Cochrane, 1971 : *Mycologia*, **63** : 462-477.

Collins, R.P. and R.D. Hackbarth, 1962 : *Mycologia*, **54** : 299-304.

Cook, R.J. and Flentze, N.T., 1967 : *Phytopathology,* 57 : 178-182.

Cook, R.J. and Papendick, R.I., 1972 : *Ann. Rev. Phytopatho.*, 349-370.

Dahl., A.S., 1934 : *Phytopathology,* **24** : 197-214.

Dale, E., 1912 : *Ann. Mycol., Barl.*, **10** : 462-477.

Dale, E., 1914 : *Ibid.* **12** : 33-62.

Datta Pranab and B.C., Das, 2002 : Management of collar rot of tomato by *Trichoderma* sp. and chemicals. *Indian Phytopath.*, **55** : 235-237.

Dayal, R. and M.M. Joshi, 1969 : *Nova Hedwigia,* **17** : 59-63.

Dhingra, D.D. and J.B. Sinclair, 1975 , *Phytopathology*, **65** : 236-240.

Durrell, L.W. and L.M. Shielde, 1960 : *Mycologia,* **52** : 636-641.

Dwivedi, R.S. and Tandon, R.N., 1975 : *Proc. Natl. Acad. Sci.* (India) **54 (B)** : 111-116.

Dwivedi, R.S., 1965 : *Proc. Nat. Acad. Sci. India*, **35 (B)** : 255-274.

Dwivedi, R.S., 1966 : *Trop. Ecol.*, **7** : 84-99.

Dwivedi, R.S., 1979 : Biology of rhizosphere myco-organisms. p. 51-61. In K.S. Bilgrami (ed.), Bio Microorganisms Vol. I, S.B. Saxena Commem. Volume.

Dykstra, T.F., 1961 : Production of disease-free seed, *Bot. Rev.*, **27** : 445.

Edson, H.A. and M. Shapolov, 1920 : *Jour. Agric., Research*, **18** : 511-524.

Elliott, E.S., 1949 : *Proc. West. Va. Acad. Sci.*, **20** : 65-68.

Ellis, M.A. and Paschal, E.H., II, 1977 : Methods of controlling internally seed-borne fungi of pigeonpea (*Cajanus cajan*). *Proceddings of the American Phytopathological Society*, **4** : 176 (Abstract).

Ellis, M.A., Paschal, E.H., II and Ravalo, E.J. and Rosario, E., 1978 : Effect of growing location on internally seed-borne fungi, seed germination and field emergence of pigeonpea in Puerto Rico. *Journal of Agricultures* of the University of Puerto Rico, **62** : 355-360.

Esposito, R.G., H. Greenwood and A.M. Flitcher, 1962 : *J. Bacteriol.*, **83** : 250-255.

Eveleigh, D.E., 1961 : *Ann. Appl. Biol.*, **40** : 412-423.

*Fitzpatrick, E.A., (1980) : Soils ; The formation, classification and distribution. Longman, London, pp. 353.

Flor, H.H., 1955 : *Phytopathology*, **39** : 172.

Fries, N., 1948 : *Hereditas*, **34** : 338-350.

Fries, N., 1965 : In B.C. Ainsworth and A.S. Sussaman, The Fungi, An Advance treatise, Vil. I pp. 491-523.

*Funk, C., 1914 : Die Vitamine. J.F. Bergman Wiesbandend.

Galloway, L.D., 1936 : *Indian J. Agric. Sci.*, **6** : 578-585.

Games, W. and Domesh, K.H., 1969 : *Trans. Brit. Mycol., Soc.*, **52** : 301-308.

Garber, E.D., 1956 : A nutrition inhibition hypothesis of pathogenicity, Am. Naturalist, **40** : 183-194.

Garrett, S.D., 1938 a : *Biol. Rev.*, **13** : 159-185.

Garrett, S.D., 1938 b : *Ann. Appl. Biol.*, **25** : 742-766.

Garrett, S.D., 1956 : Biology of root infecting fungi. Cambridge University Press, London.

Garrett, S.D., 1965 : "Toward biological control of soil borne plant pathogens" in Ecology of soil borne plant pathogens, K.F. Barker and W.C. synder (eds.) University of California Press, Barkley, California 4-17.

Garrett, S.D., 1967 : *Trans. Brit. Mycol. Soc.*, **50** : 519-524.

Gaumann, E., 1957 : *Phytopathology*, **47** : 342-357.

Gaumann, E., S. Naefroth and H. Kobel., 1952 : *Phytopathol. Z.*, **20** : 1-38.

*George, L.K., 1951 : *Mycologia*, **43** : 297-309.

Ghatak, P.N. and T.C. Roy, 1939 : *J. Ind. Bot. Soc.*, **18** : 113-127.

Ghosh, A.K., 1964 : D. Phil. Thesis, University of Allahabad.

Goldstrohm, D.D., 1963 : *Proc. West Viriginia Acad. Sci.*, **35** : 33-36.

Gottlieb, D., 1964 : *Endeavour.*, **23** : 85-89.

Gough, F.J. and V.G. Lilly, 1956 : *Proc. West Virginia Acad. Sci.*, **27** : 19-24.

Graf, G., 1930 : *Centbl. Bakt.*, **88** : 44-69.

Gray, P.H.H. and C.H. Taylor, 1935 : *Thid., C.* **13** : 251-255.

Gray, P.H.H. and N.B. Mc. Master, 1933 : *Canad. J. Res.* **8** : 375-389.

*Griffin, D.M., 1963 a : *Biol. Rev.*, **38** : 141-166.

*Griffin, D.M., 1963 b : *Trans. Brit. Mycol. Soc.*, **46** : 373-377.

*Griffin, D.M., 1969 : Soil water in ecology of fungi. *Ann. Rev. Phytopath.*, **7** : 289-310.

*Griffin, D.M., 1972 : Ecology of soil fungi. Syracuse University Press, pp. 193.

Gupta, R.B.L., B.G. Desai and V.N. Pathak, 1970 : *Nova Hedwigia*, **19** : 349-350.

*Hackborth, R.D. and R.P. Collins, 1961 : *Am. J. Bot.*, **48** : 603-606.

*Hagem, C., 1907 : Untersuchungen Uber Norwegische Mucorinsen. I. Vid-Selsk, S. Kr., *Math. Naturv. Klasse* **7** : 1-50.

Lal, B., 1986 : *Proc. Natl. Acad. Sci., India*, **38 B** : 1-8.

Harman, G.E., Chet, I. and Baer, R., 1981 : Factors affecting *Trichoderma hamatum* applied to seed as a biocontrol agent. *Phytopathology,* **71** : 569-572.

*Harvey, J.V., 1925 : *J. Elisha Mitchell Sci. Soc.*, **41** : 161-164.

Hasan, A. and Khan, A.M., 1979 : Varietal screening for anthracnose disease of arhar, *Act.-Botanica-India,* **7 (2)** : 126-128.

Hasija, S.K., 1970 : *Mycologia,* **62** : 289-295.

Hawker, L.E., 1938 : *Nature*. London, **142** : 1039.

Hawker, L.E., 1950 : Physiology of Fungi, University of London, Press Ltd., London, pp. 360.

Hedge, R.K. and K.G. Ranganahiiah, 1971 : *Mysore J. Agric. Sci.*, **5** : 101-104.

*Hiltner, L., 1904 : Uber Neuere Erfahrungen and probleme auf dem Gebiet der Bodenbakteriologie and unter besonderer Beruck sichtigung der groundungung und Brachae, Arb. dtsch. Londwn Ges, **98** : 57-78.

Hine, R.B. and M. Agragaki, 1963 : *Phytopathology*, **53** : 1194-1197.

Horsfall, J.G., Heuberger, J.W., Sharvell, E.G. and Hamilton, J.M., 1940 : *Phytopathology,* **30** : 545-563.

Husain, S.S. and Ahmed, M.A., 1971 : Studies on stored food grain fungi part-II Fungi from oil seeds and *Plantago ovata. Pakist, J. Scient. Ind. Res.*, **14 (1-2)** : 137-141.

Illyas, M.B., M.A. Ellis and J.B. Sinclair, 1975 : *Pl. Dis. Rept.*, **59** : 360-364.

Issac, I., 1956 : *Ann. Appl. Biol.*, **44** : 105-112.

ISTA, 1985 : International Rules of seed testing, *Seed Sci. and technology*, **13** : 299-355.

Jackson, M.L., 1973 : Soil chemical analysis, Prentice Hall of India Ltd., New Delhi, 115-151.

Jamaluddin, 1973 : D. Phil. Thesis, University of Allahabad.

Jensan, C.N., 1912 : *Bull. Cornell Agric. Exp. Sta.*, **315** : 414-501.

Joffe, A.Z. and J. Palti, 1972 : *Phytopath. Z.*, **73** : 123-148.

Johnson, J.W., 1923 : *Iowa Agric. Expt. Res. Bull.*, **76** : 308-342.

Johnson, L.F. and E.A., Curl, 1972 : Methods for Research on the ecology of soil borne plant pathogens, Burgess Publishing Company, Minneapolis, Minnesota, p. 247.

Joshi, L.K. and M.P. Haware, 1966 : *Indian Phytopath.*, **10** : 168-172.

Kaiser, W.J., 1964 : *Phytopathology*, **54** : 481-487.

Kakkar, R.K., 1964 : D. Phil. Thesis, University of Allahabad.

Katznelson, H., 1965 : Nature and importance of rhizosphere. pp. 187-209. In : Ecology of soil borne plant pathogens (eds. K.F. Baker and W.C. Synder), University of California Press, Berkeley.

Khanna, K.K. and Chandra, S., 1972 : *Proc. Nat. Acad. Sci., India*, **42 (B)** :

Khanna, K.K., 1974 : D. Phil. Thesis, University of Allahabad.

Klisiewicz, J.M., 1963 : *Phytopathology*, **53** : 1046-1059.

Kommedahl, Thor, Christensen, J.J., Culberton, J.O. and Moore, M.B., 1955 : Univ. Minnesota Agri. Exp. Sta. Tech. Bull., 215-240.

Kumar, K. and Patnaik, 1985 : Seed-borne nature of *Alternaria alternata* in pigeonpea, its detection and control, *Indian J. Pl. Pathol.*, **3** : 69-73.

Kuo, M.S. and R.P. Scheffer, 1964 : *Phytopathology*, **54** : 1041-1044.

La Touche, C.J., 1948 : *Nature*, London, **161** : 320.

Lakshminarayanan, K. and D. Subramanian, 1955 : *Nature*, London, **176** : 697-698.

*Lendner, A., 1908 : Materiaux Pour la Flore Cryptogramique Suisse, III, Fasc. I.

Leonian, L.H. and V.C. Lilly, 1942 : *Am. J. Bot.*, **29** : 459-464.

Leonian, L.H. and V.G. Lilly, 1938 : *Phytopathology*, **28** : 531-548.

Lilly, V.G. and H.L. Barnett, 1947 : *Am. J. Bot.*, **34** : 131-138.

Lilly, V.G. and H.L. Barnett, 1949 : *W. Va. Univ. Bull. Ser.*, **49** : No. 9-11, 69-74.

Lilly, V.G. and H.L. Barnett, 1951 : *Physiology of the Fungi*, Mc Graw Hill Book Co. Inc., New York, p. 464.

*Lindeberg, G., 1946 : *Botan. Notiser*, **99** : 89-93.

Link, K.P. and Walker, J.C., 1933 : *J. Biol. Chem.*, **100** : 379-383.

Lokesh, M.S., R.V. Hirenath and R.K. Hodge, 1987 : Seed mycoflora of red gram (*Cajanus cajan L.)* Mill. sp. *Pl. Path. News letters*, **5** : 31.

*Machacek, J.E. and Greany, F.J., 1933 : *Can. J. Res.* **8** : 276-281.

Martin, J.P., 1950 : Use of acid rose bengal and stretptomycin in the plate method for estimating soil fungi, *Soil Sci.*, **60** : 215-232.

Massey, L.M., 1926 : *Phytopathology*, **16** : 509-523.

Mathew, K.T., 1952 : *Nature*, London, **170** : 889.

Mathew, K.T., 1953 : *Proc. Indian Acad. Sci.*, **38 B** : 1-10.

Mathur, B.L., L.C. Sharma and N. Prasad, 1964 : *Indian Phytopath.*, **27** : 121-125.

Mathur, R.S., H.L. Barnett and V.B. Lilly, 1950 : *Phytopathology*, **40** : 104-114.

Meachum, M.R., 1918 : *Science*, **48** : 499-500.

Mehta, P.P. and R.K.P., Sinha, 1982 : A new leaf-spot diseases of Arhar (*Cajanus cajan* L.) from India, *Sci. & Cult.*, **48** : 44.

Mishra, K.P., 1992 : Studies on pathogenic fungi of fruit trees. D. Phil Thesis, University of Allahabad.

Misra, A.P. and M. Mahmood, 1961 : *Indian Phytopath.*, **14** : 20-22.

Misra, S.B., 1975 : D. Phil. Thesis, University of Gorakhpur.

Misra, S.B., Misra, R.R. and Dixit, S.N., 1974 : *Proc. Nat. Acad. Sci. India*, **76 (B)** : 174.

Moore, E.S., 1924 : *Ann. Bot.*, **38** : 137-161.

Muller, H.J., 1962 : *Phytopath. Z.*, **44** : 381-412.

Mundkur, B.B., 1936 : Resistance of American Cottons to Fusarium wilt in India, *Proc. Indian Acad. Sci.*, **3 B** : 498-501.

Muskett, A.E. and Malone, J.P., 1941 : *Ann. Appl. Biol.*, **28** : 8-13.

Nene, Y.L. and A.K. Agarwal, 1978 : Some seed-borne diseases and their control, ICAR, New Delhi, 44.

Nene, Y.L., 1985 : A proposed list of common names for diseases of pigeonpea, *International Pigeonpea News letter*, **4** : 31-32.

Nicholas, D.J.D., 1965 : Influence of the rhizosphere on the mineral nutrition of the plant. pp. 210-217. In : Ecology of soil borne plant pathogens (eds.) K.F. Baker and W.C. Synder), John Murray, Albemarie Street, London.

Nishimura, S., 1957 : *Ann. Phytopathol. Soc., Japan*, **22** : 274-275.

Noble, M. and Richardson, M.J., 1968 : *Proc. Int. Seed Test, Ass.* **33** : 1-191.

Noble, M., 1951 : Seed pathology, *Nature*, London, **168** : 534.

Noble, M., 1957 : The transmission of plant pathogens by seed, C. Horton-Smith (Ed.) Acadmic Press, New York, **1** : 195-237.

Noble, M., J. De Tempe and P. Neergaard, 1958 : An Annoted list of seed-borne diseases, common wealth mycological institute, Kew, Survey England.

Noecker, N.L. and M. Reed, 1943 : *Am. Midland Naturalist*, **30** : 171-174.

Nolan, R.A., 1970 : *Mycologia*, **62** : 568-577.

Norton, D.C., 1953 : *Phytopathology*, **43** : 633-636.

Paharia, K.D., 1983 : Plant protection and pesticide use in Madhya Pradesh, *Plant protection, Bulletin, India*, **35** : 1-2, 2-4.

Papavizas, G.C., 1967 : Evaluation of different media and anti-microbial agents for isolation of fungi, *Soil Sci.*, **88** : 112-117.

Papavizas, G.C., 1970 : *Mycologia*, **62** : 1195-1203.

*Pasteur, L., 1860 : Note relative on *Penicillium glaucum* et a la dissymetric molecuaire des produits organiques naturals, *Comt. Rend. Acad. Sci.*, **51** : 298-299.

*Pehrson, S.O., 1948 : *Physiologia Plantarum*, **1** : 38-56.

Peterson, E.A., 1959 : *Con. J. Microbiol.* **5** : 579-582.

Phanasenko, V.T., 1967 : *Bot. Rev.*, **33** : 189-225.

*Poschenriedder, H., 1930 : Uber die varbreitung des azotobakter im wurzelbere iche der pflanzen. *Cantbl. Bakt.*, **80** : 369-378.

Prasad, S.S., 1966 : *Phytopath. Z.*, **55** : 20-25.

Rajan, P.P., S.R. Gupta, Y.R., Sarma, and G.V.H. Jackson, 2002 : Diseases of ginger and their control with *Trichoderma harzianum. Indian phytopath.*, **55 (2)** : 173-177.

Rajkule, P.N., Goyal, S.N. and Vala, D.G., 1989 : Screening of pigeonpea varieties against *Fusarium* wilt. *Internat. Pigeonpea News letter*, **9** : 24.

Ramkrishnan, T.S., 1942 : *Proc. Ind. Acad. Sci.*, **14 B** : 395-411.

Raper, K.B. and C. Thom, 1949 : Manual of the *penicillia*, The williams and wilkins co., Baltimore, Madison.

*Raulin, J., 1869 : Etudes chimiques sur vegetation, *Ann. Sci. Nat., Botan.*, **11** : 93-229.

Reddy, M.V., K.B., Saxena, Onkar Singh, R.S.L. Karna, R. Sahu, M.P. Bharti and R.P. Sah, 1989 : Macrophomina Stem canker of pigeonpea in Nepal, *International Pigeonpea News letter*, **9** : 22-23.

*Remsberg, R.B., 1940 : *Mycologia*, **32** : 52-56.

Riker, A.J. and R.S. Riker, 1936 : Introduction to research on plant disease John S. Swift Co., St. Loius.

Robbins, W.J. and F. Kavanagh, 1938 : *Am. J. Bot.*, **25** : 229-236.

Robbins, W.J. and R. Ma, 1942 : *Bull. Torrey Bot. Club.*, **69** : 184-203.

Robbins, W.J. and V. Kavanagh, 1942 : Bot. Rev., 8 : 411-471.

Ross, D.J., 1960 : *N.Z.J. Sci.*, **3** : 219-257.

Rouatt, J.W. and H. Katznelson, 1961 : *J. App. Bacteriol.*, **24** : 164-171.

Rovira, A.D. and C.B. Davey, 1974 : Biology of the rhizosphere, pp. 153-204. In the plant roots and its environment (ed. E.W. Carson), University Press of Virginia, Chartottesville.

Rovira, A.D., 1965 : *Ann. Rev. Microbiol.*, **19** : 241-266.

Roy, R.Y., R.S. Dwivedi and P.N. Singh, 1980 : *Acta. Botanica Indica.*, **8** : 23-30.

Rudolph, B.A. and Harrison, G.J. 1945 : *Phytopathology*, **35** : 542-548.

Sadasivan, T.S. and C.V. Subramanian, 1954 : *Trans. Brit. Mycol. Soc.*, **37** : 426-430.

Saharan, G.S. and Gupta, V.K., 1974 : Indian I. Myceal *Plant Path.* **3** : 222-224.

Saksena, R.K. and B.S. Mehrotra, 1952 : *Proc. Natl. Acad. Sci. India*, **22 B**:22-43.

Saksena, R.K. and D. Kumar, 1962 : *Proc. Natl. Acad. Sci., India*, **32 B** : 37-49.

Saksena, R.K., 1958 : *Memoris Ind. Bot. Soc.*, **1** : 71-76.

Sandhu, R.S., 1960 : *Phytopathology. Z.*, **37** : 33-60.

Sarbhoy, A.K., 1963 : D. Phil. Thesis, University of Allahabad.

Satyaprasad, R., 1982 : *Indian Phytopath.*, **35** : 153-154.

Saxena, H.K., K. Kumar, 1971 : Some aspects of epidemiology and control of phyllosticta leaf-spot of arhar (*Cajanus cajan*) L. Milbp.), In (Raychaudhuri, S.P. (Convener) : Epidimiology and control of plant diseases. Proc. Ind. Nat. Sci. Acad., B. 1971, Publ. 1972 : 1973, **37** : 309-406.

*Schopfer, W.H., 1935 : *Z. Vitaminforech.*, **4** : 187-206.

*Schopfer, W.H., 1943 : Plant and Vitamins, Chronica Botanica Co., Waltham, pp. 293.

Sekhawat, P.S. and Prasad, R., 1971 : *Indian Phytopath.*, **24** : 800-802.

Selvaràjan, R., 1990 : Biological control of chickpea root-rot caused by *Fusarium solani* (Mart.) Sacc. and *Macrophomina phaseolina* (Tassi.) M. Sc. (Ag.) Thesis, Tamil Nadu Agri. University Coimbatore, India, p. 115.

Sen Gupta, P.K. and S. Roy, 1971 : *Z. Fur. Pflkrankhund Pflan.*, **78** : 670-674.

Shaw, B.T., 1952 : Soil Physical conditions and plant growth. Agron. Monograph 2, Academic Press, New York, p. 491.

*Shaw, F.J.F., 1915 : *Pusa Annu. Rep.*

*Sherwood, E., 1923 : *Am. J. Bot.*, **10** : 537-553.

Shiv Kumar, 1975 : D. Phil. Thesis, University of Allahabad.

Shukla, D.N. and Bhargava, S.N., 1977 : Fungi isolated from seeds of pulses and oil crops stored for different intervals of time, *Vigyan Parishad Patrika*, **2** : 363-367.

Shukla, D.N., 2000 : Biological relationship and effect of environmental factors on disease development of Pulses and oil crops. D.Sc. Thesis, University of Allahabad, Allahabad.

Singh, B.B., Singh, D.P., Maurya, D.M., Kumar, R., Rai, O.P., Gupta, R.P., 1998 : Narendra Arhar-1 : a high yielding, disease-resistant, long duration pigeonpea cultivar for Uttar Pradesh, India. *International Chikpea and pigeonpea. News Letter*, **5** : 33-34.

Singh, H.B., 1988 : Leaf Blight of pigeonpea by *Fusarium semitectum*, *International pigeonpea News letter*, **7** : 29-30.

Singh, M.K. and Prasad, T., 1977 : *Indian Phytopath.* **30** : 70-72.

Singh, Narendra and Singh, R.S., 1983 : Inhibition of *Fusarium udums* (pigeonpea wilt pathogen) by other distillate of Margosa cake amended soil. *Indian J. Mycol. Pl. Pathology*, **23** : 329-333.

Singh, S.P., Jain, H.K., Ramanujam, S., Hayat, Ram, Pahuja, A.N., Govil, P.N., 1988 : Pusa 74 a high yielding arhar variety. *Indian Farmeing*, **37** : 12,9-10.

Sinha, P. and Saxena, S.K., 1987 : Effect of neem leaf powder and extract on the development of fruit rot caused by *Aspergillus niger. Neem News* (1) : 45-47.

Sivan, A.A., Y. Elad and I. Chet., 1984 : Biological control effects of a new isolate of *Trichoderma harzianum on pythium aphanidermatum. Phytopathology*, **74** : 498-501.

Srinivasapai, K.V., 1953 : *Proc. Indian Acad. Sci.*, **37 B** : 131-148.

Srivastava, J.N., 1996 : Biological control of pulse disease. D. Phil Thesis, University of Allahabad.

Srivastava, M.P. & Tondon, R.N., 1977 : *Indian phytopath.*, **24** : 396-397.

Stanley, J.R. and J.A. Nimmo, 1979 : *Phytopathology*, **50** : 496-498.

Subramanian, C.V., 1951 : *Nature*, London, **168** : 561.

Subramanian, C.V., 1952 a : *Proc. Natl. Inst. Sci. India*, **18** : 557-558.

Subramanian, C.V., 1952b : *J. Madras Univ.*, **22 B** : 206-222.

Suryanarayan, D., Bhombe, B.B. and Ram Nath, 1963 : Testing of vegetable seeds for freedom from diseases, *Indian Hort.*, **20** : 141-145.

Suryanarayana, D., 1978 : Seed pathology, Vikas, New Delhi, p. 101.

Suryanarayanan, S., 1958 : *Proc. Ind. Acad. Sci.*, **48 B** : 154-188.

Tandon, M.P., 1950 : D. Phil. Thesis, University of Allahabad.

Tandon, R.N. and K.S. Bilgrami, 1957 c : *Proc. Natl. Acad. Sci., India*, **27 B** : 98-105.

Tandon, R.N. and S.N. Bhargava, 1960 : *Proc. Natl. Acad. Sci., India*, **30 B** : 257-265.

Tandon, R.N., 1961 : Physiological studies on some pathogenic fungi, Scientific Research Committee, Uttar Pradesh, Allahabad. India, pp. 80.

Thakur, A.K. and R.V. Norris, 1928 : *J. Indian Inst. Sci.*, **11 A** : 141-160.

Timonin, M.I., 1940 : *Can. J. Res. Sec.*, **18 B** : 444-456.

Tisdale, W.H., 1917 : *Phytopathology*, **7** : 256-360.

Togashi, K., 1949 : Biological Characteristics of Plant Pathogens Temperature Relations. Tokyo : Meibundo, pp. 478.

Toussoun, T.A., V. Bega, Robert and E. Nelson Paul, 1970 : Root disease and soil-borne pathogens, Univ. of Calf. Press, Berkeley, Los Angeles and London.

Tresner, H.D., M.P. Rackus and J.T. Curtis, 1954 : *Mycologia*, **46** : 314-333.

Tripathi, R.D., Srivastava, H.S. and Dixit, S.N., 1978 : Fungitoxic principle from the leaves of *Lawsonia inermis* Lam. *Experientia.* **34** : 51-52.

Upadhyay, R.S. and Rai, B., 1979 : *Rev. Ecol. Biol. Sol.*, **16** : 39-40.

Vail., W.J., 1962 : *Proc. West. Virginia Acad. Sci.*, **34** : 32-34.

*Valaskova, E., 1962 : *Acta Pruhonic*, 61-71.

Venkat Ram, 1954 : Ph.D. Thesis, University of Madras.

Wadhwani, K., Mehrotra, N. and Dudeja, S.K., 1986 : The effect of some plant extract, on uredospore germination of *Paccinia helianthi. Journal of Palynology.* **22** : 133-136.

Wallen, V.R., 1964 : Host-parasite relations and environmental influences in seed-borne diseases, Microbial behaviour, symposia of the society for general Microbiology, Number XIV, pp. 187-212.

Warcup, J.H., 1951 : *Trans. Brit. Mycol. Soc.*, **34** : 376-399.

Warcup, J.H., 1955 : *Nature*, London, **175** : 953-954.

Ward, E.W.B., 1962 : *Can. J. Bot.*, **40** : 1347-1354.

Wicklow, D.T. and W.F. Whittingham, 1978 : *Can. J. Bot.*, **56** : 1702-1709.

Wicklow, D.T., 1973 : *Ecology.*, **54** : 1302-1310.

Wohlrab, G., R.W. Tuveson and C.E. Olmsted, 1963 : *Ecology*, **44** : 734-740.

Yabuta, T., K. Kambe and T. Hayashi, 1934 : *J. Agric. Chem. Soc., Japan,* **10** : 1059-1062.

Yabuta, T., K. Kambe and T. Hayashi, 1934 : *J. Agric. Chem. Soc.*, Japan, **10** : 1059-1068.

Yogeshwari, L., 1948 : *Proc. Indian Acad. Sci.*, **28 B** : 177-201.

Yu, Clare, C.C., 1954 : *Am. J. Bot.*, **41** : 21-30.

Zachariah, A.T., 1949 : Ph.D. Thesis, University of Madras.

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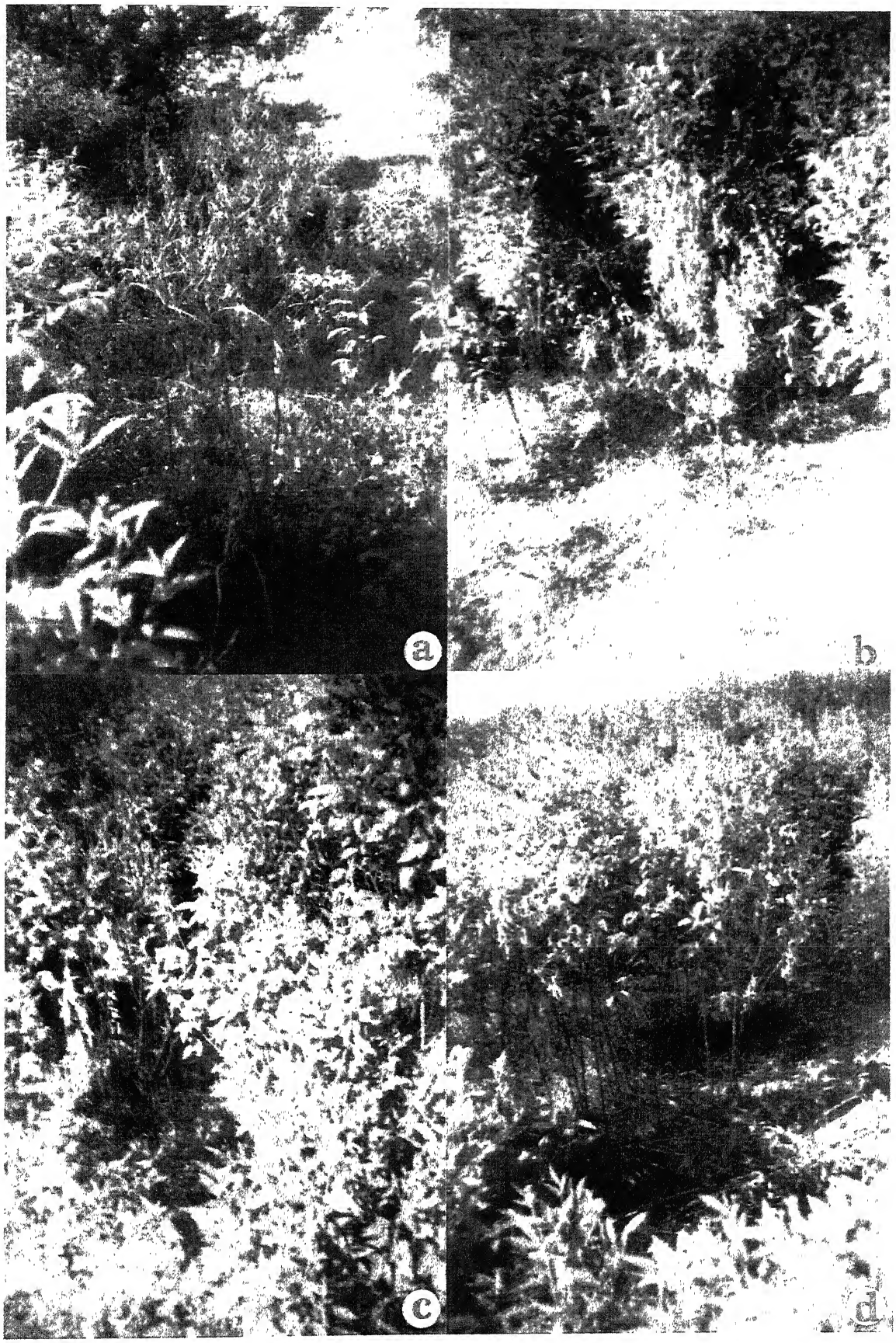
PLATE 1



Fusarial wilt of Arhar (*Cajanus cajan*)

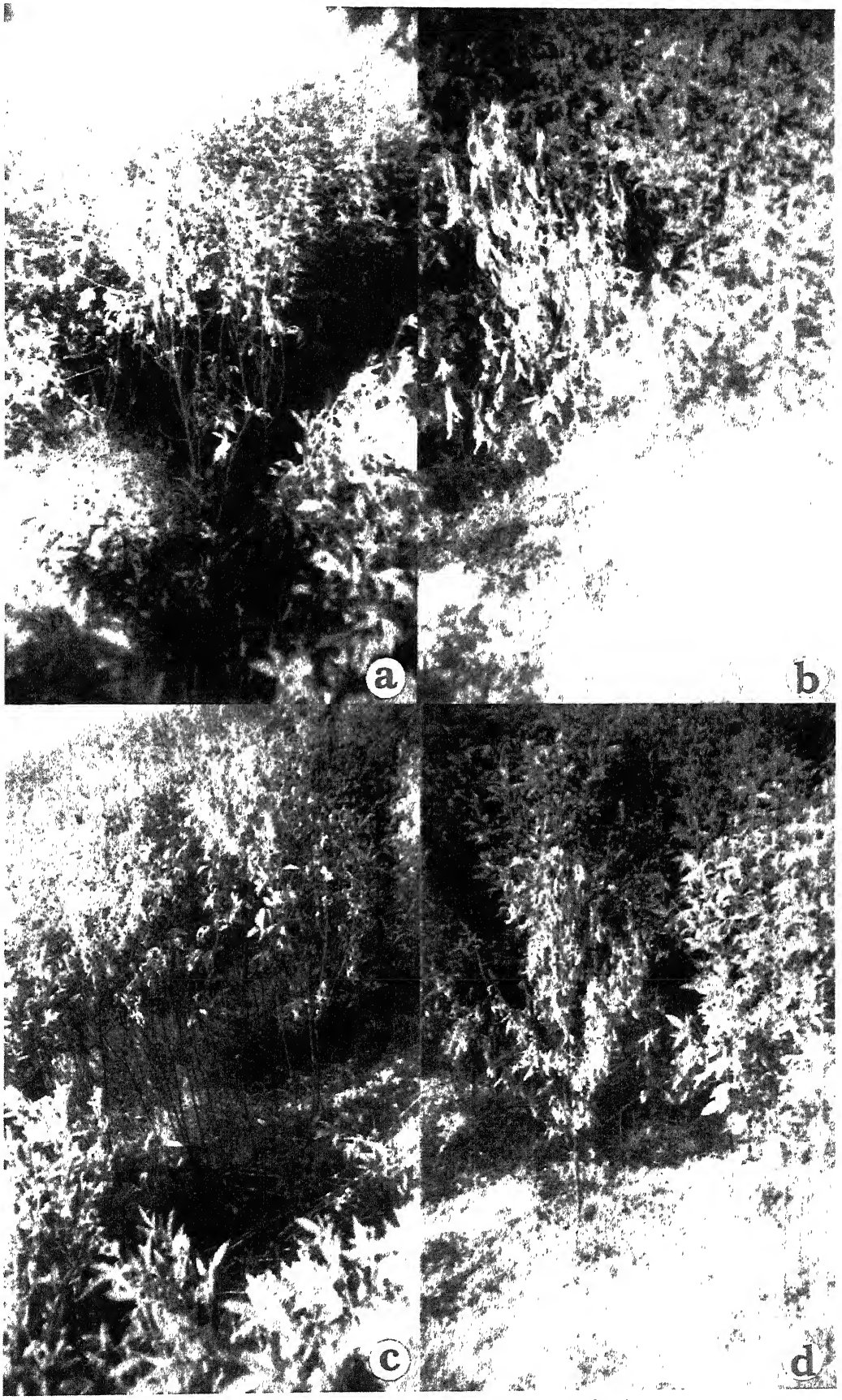
a. *Fusarium oxysporum*
b. *Fusarium solani*
c. *Fusarium solani*
d. *Fusarium* spp.

PLATE 2

Fusarial wilt of Arhar (*Cajanus cajan*)

a. *Fusarium oxysporum*
b. *Fusarium solani*
c. *Fusarium solani*

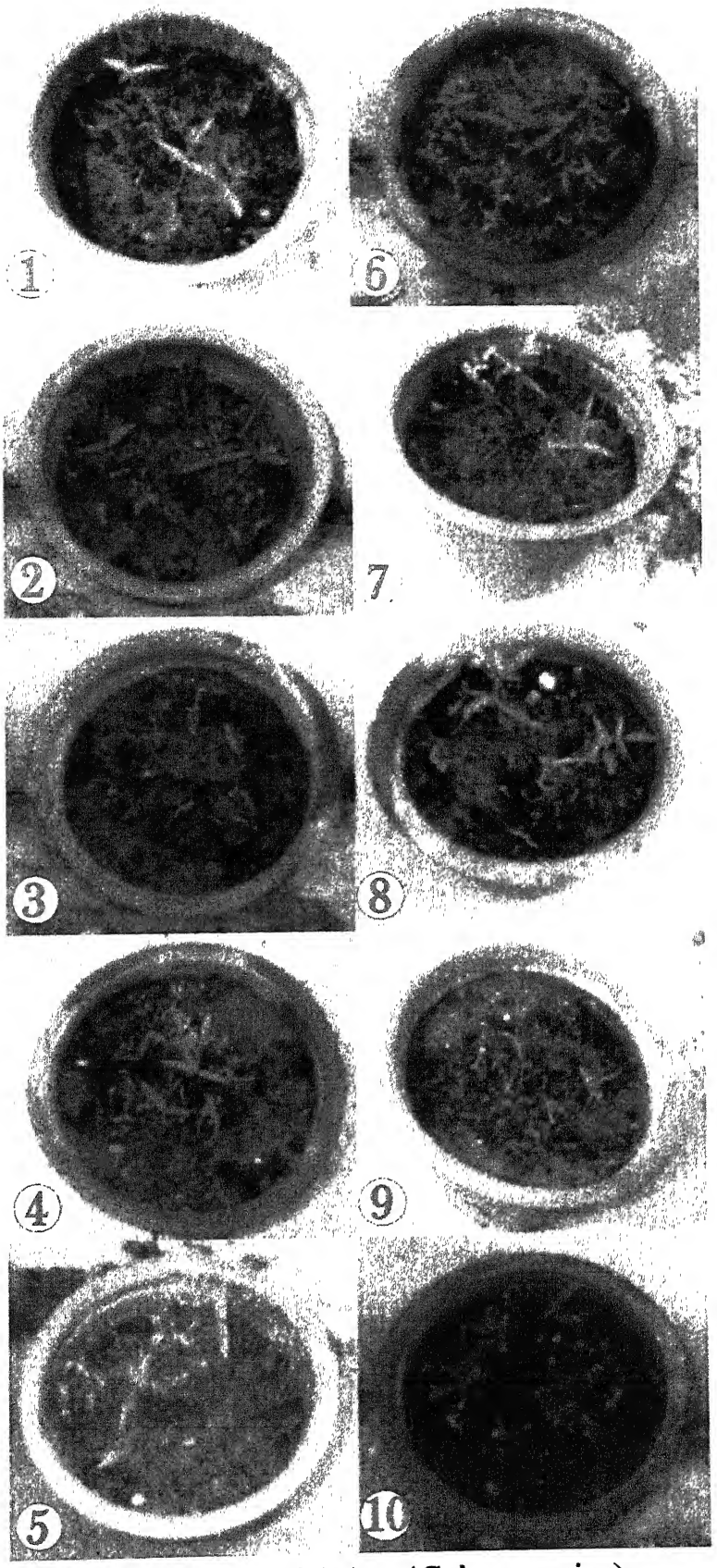
PLATE 3



Fusarial wilt of Arhar (*Cajanus cajan*)

a. *Fusarium oxysporum*
b. *Fusarium oxysporum*
c. *Fusarium solani*
d. *Fusarium* spp.

PLATE 4

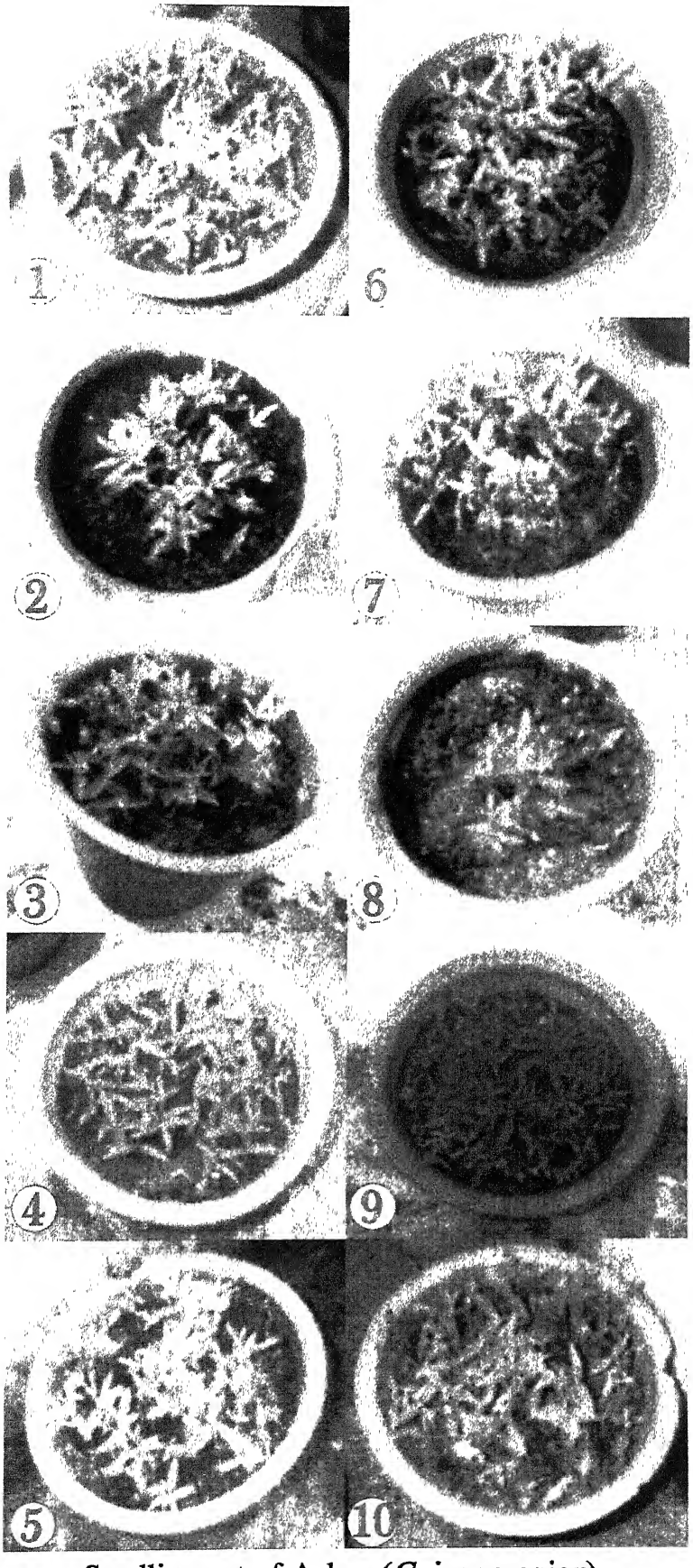


Seedling rot of Arhar (*Cajanus cajan*)
Fusarium oxysporum

Varieties:

(1) Bahar (2) GAUT 82-58 (3) Pant A8505 (4) Pant A 8508 (5) ICPL 151
(6) Prabhat (7) Pusa 18 (8) Pusa 85 (9) Pusa 604 (10) UPAS 120

PLATE 5

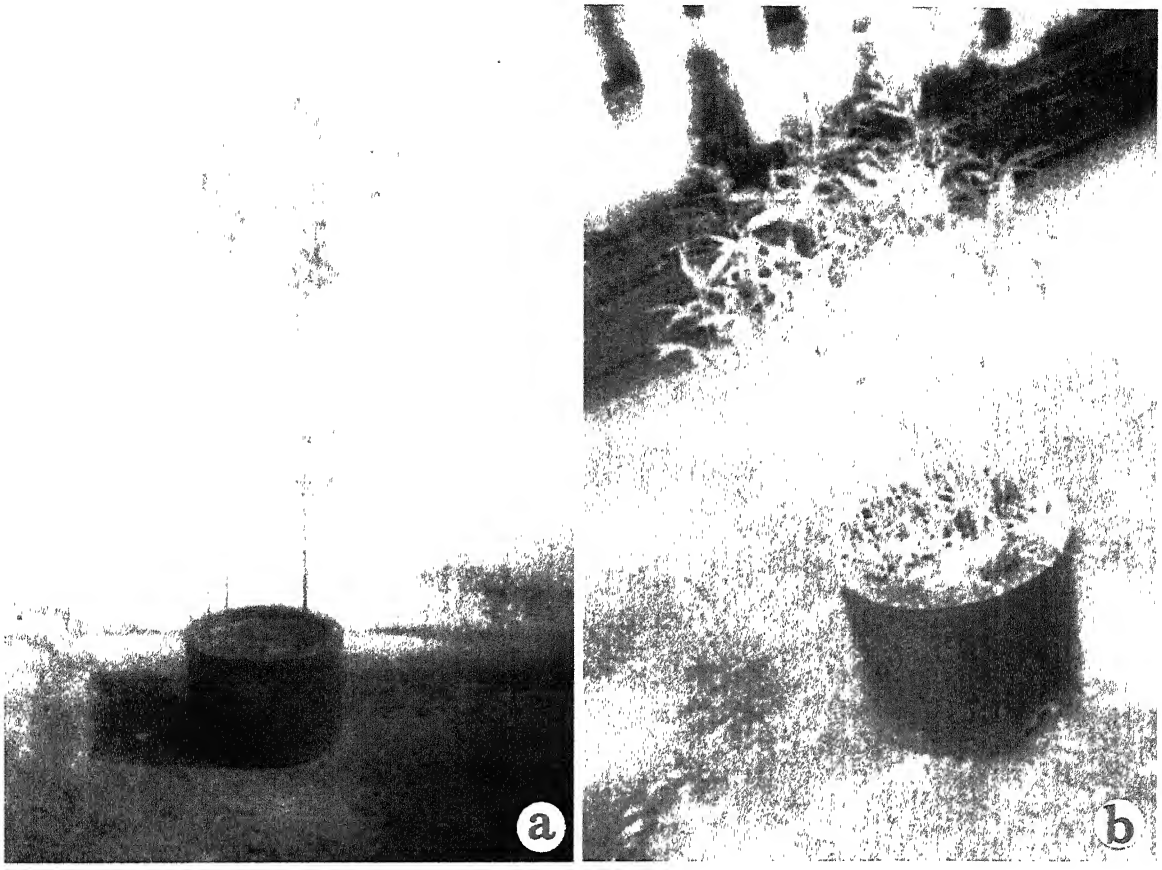


Seedling rot of Arhar (*Cajanus cajan*)
Fusarium solani

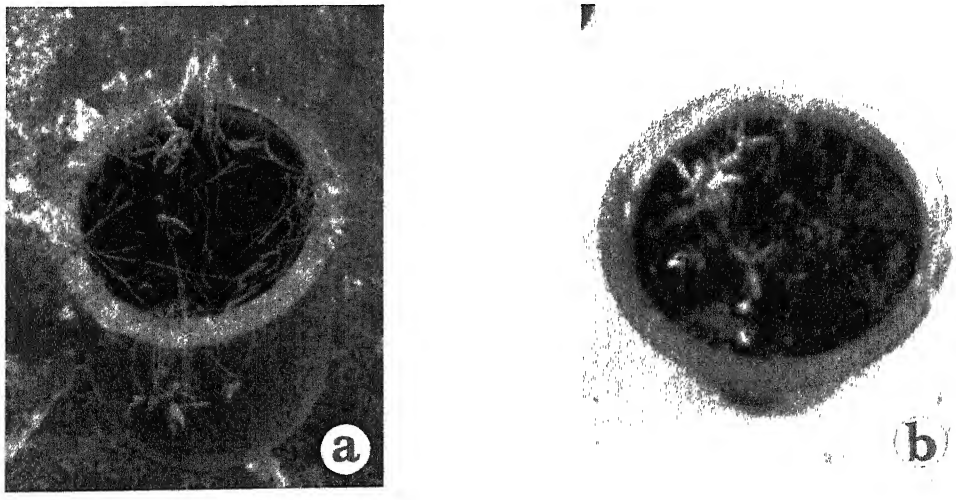
Varieties:

(1) Bahar (2) GAUT 82-58 (3) Pant A8505 (4) Pant A 8508 (5) ICPL 151
(6) Prabhat (7) Pusa 18 (8) Pusa 85 (9) Pusa 604 (10) UPAS 120

PLATE 6

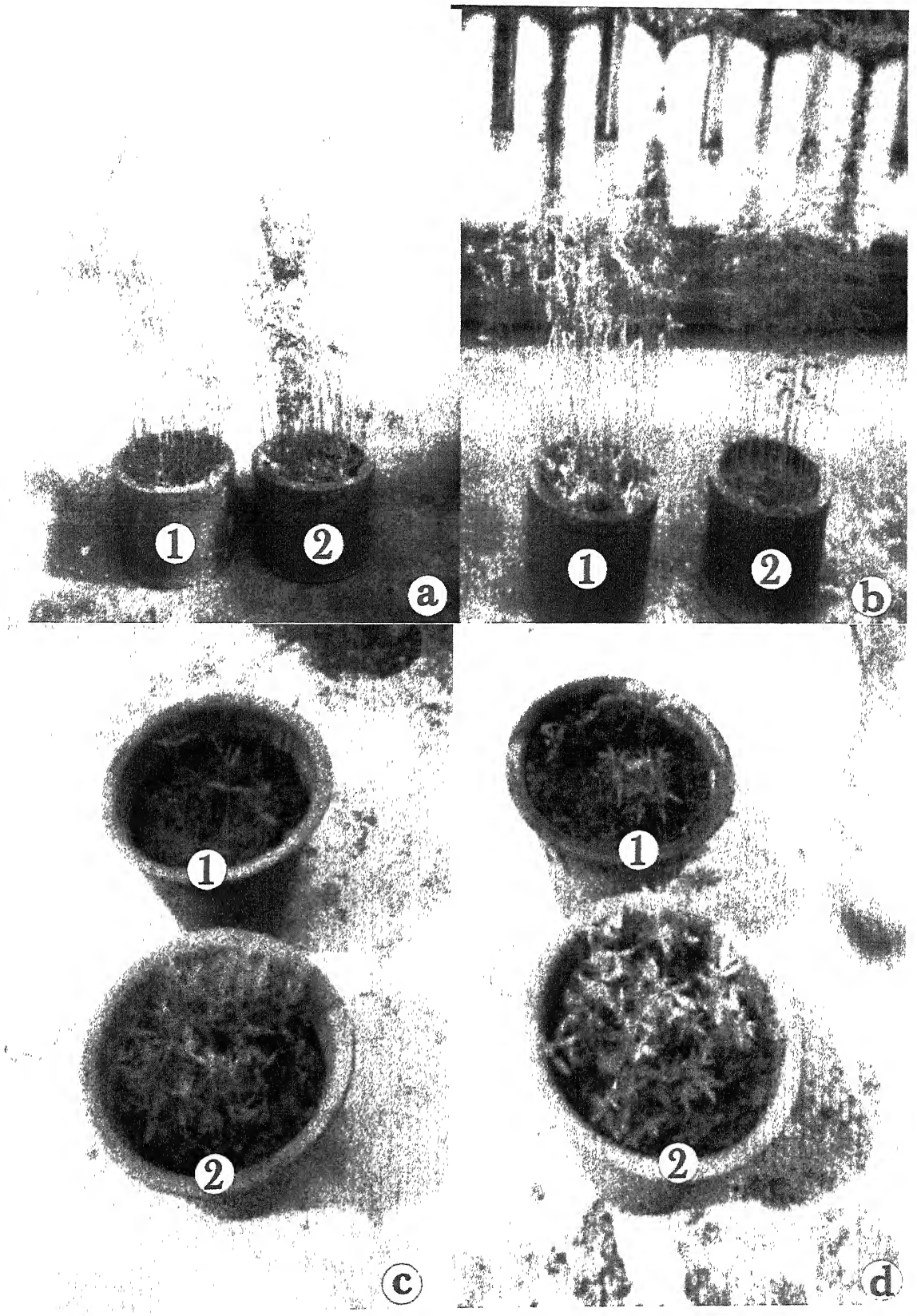


Fusarial wilt of Arhar (*Cajanus cajan*)
(a) *Fusarium oxysporum* (b) *Fusarium solani*



Seedling rot of Arhar (*Cajanus cajan*)
(a) *Fusarium oxysporum* (b) *Fusarium solani*

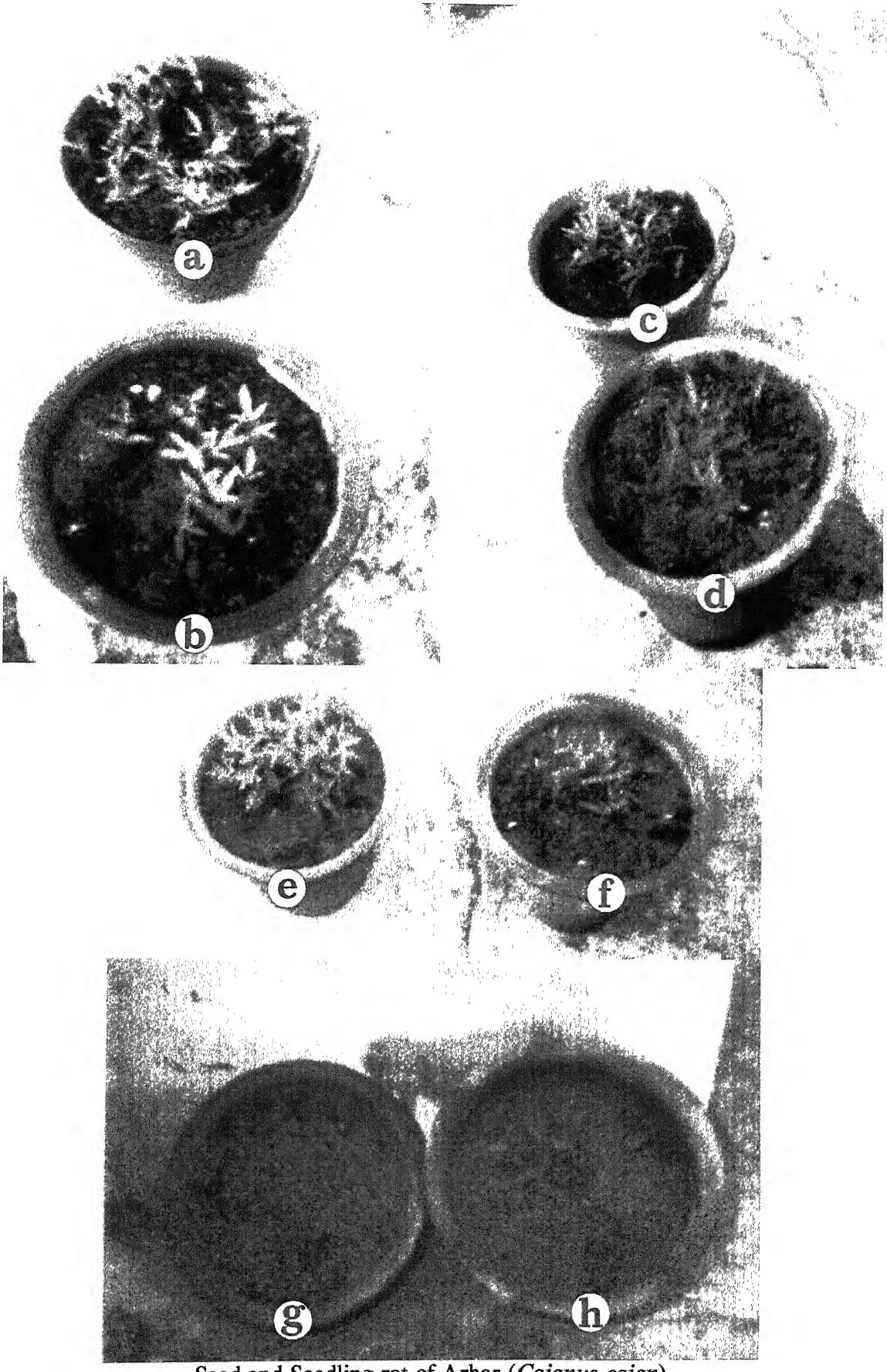
PLATE 7



Wilt and Seedling rot of Arhar (*Cajanus cajan*)
Fusarium oxysporum

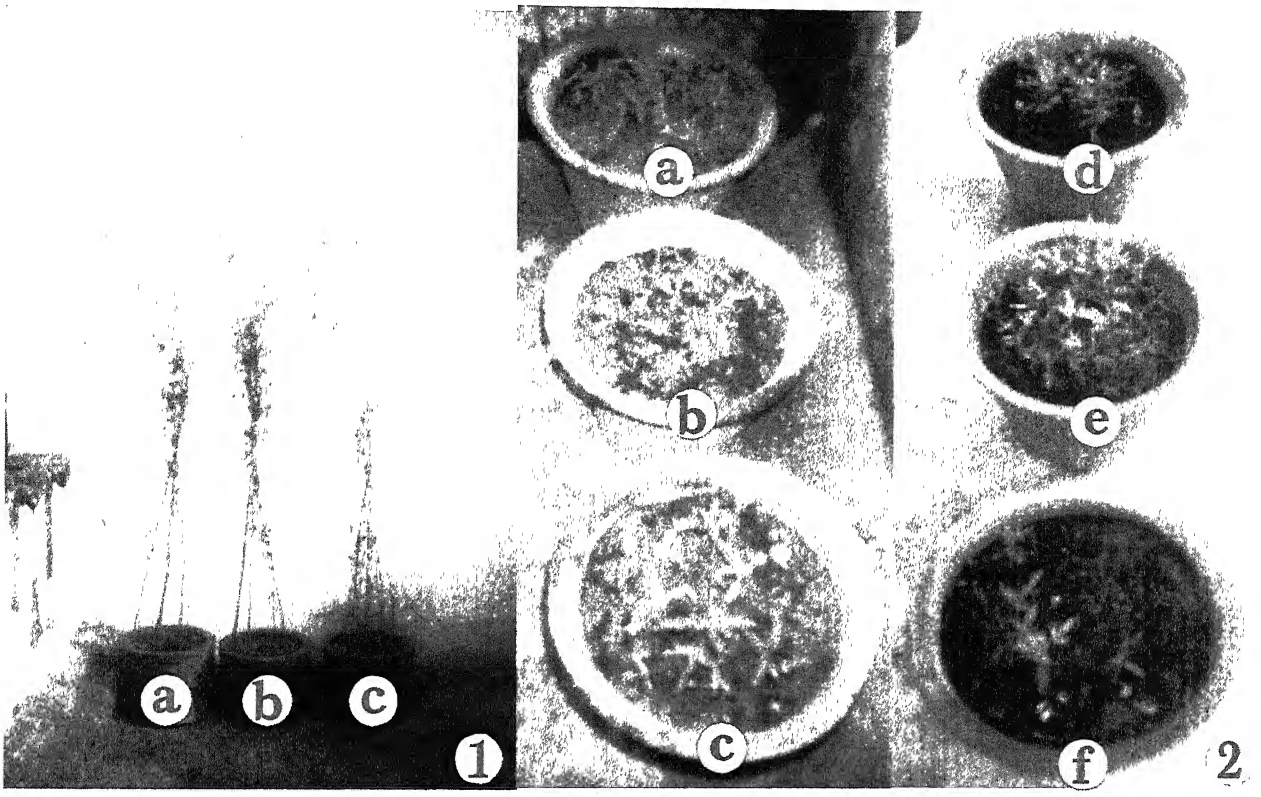
(a) Wilted [1&2] (b) 1. Wilted, 2. Control (c) Seedling rot 1. rotted, 2. control
(d) Seedling rot 1. rotted, 2. control

PLATE 8

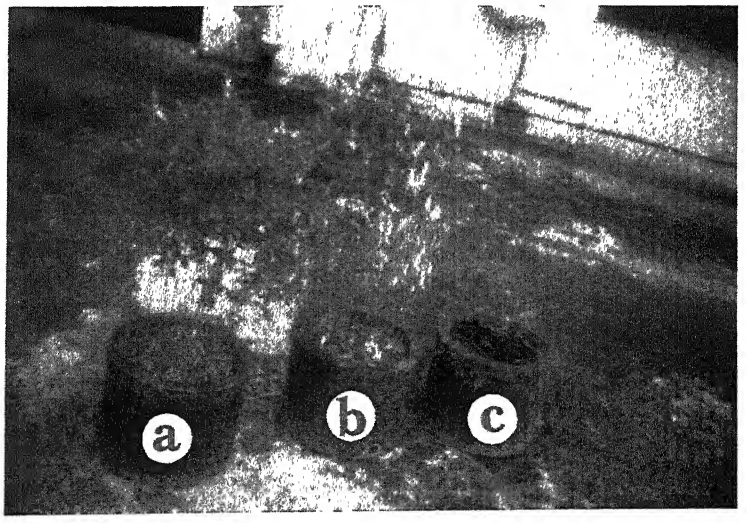


Seed and Seedling rot of Arhar (*Cajanus cajan*)
Fusarium oxysporum
Varieties : (a) Bahar (b) T21 (c) T7 (d) T17 (e) Prabhat (f) UPAS120
Seedling rot of Arhar (*Cajanus cajan*)
Fusarium solani (g) rotted (h) healthy

PLATE 9

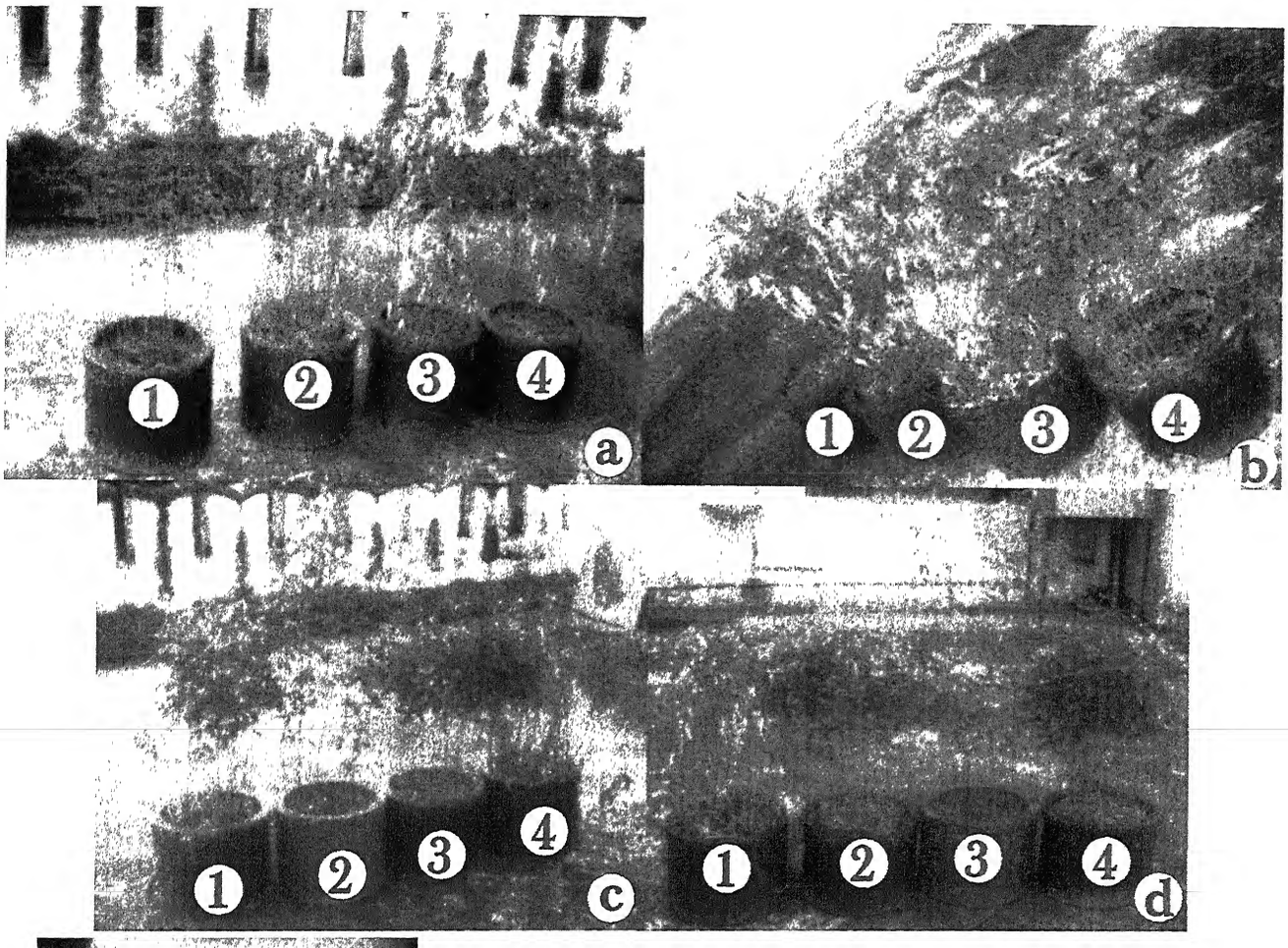


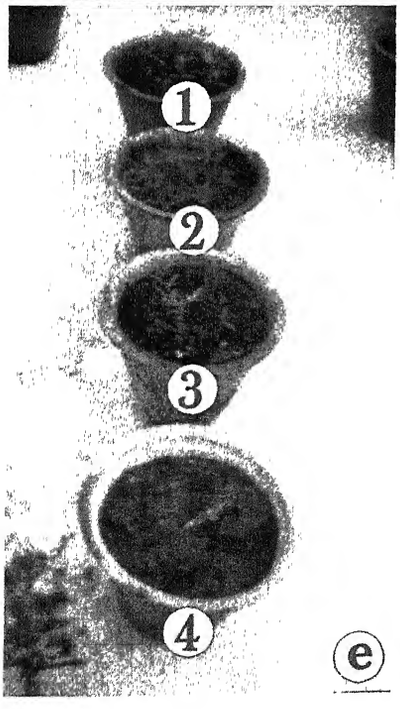
Seedling rot of Arhar (*Cajanus cajan*)
Fusarium acuminatum
Varieties: 1(a) T21, (b) Prabhat (c) UPAS120,
2 (a) GAUT 82-58 (b) GAUT 82-90 (c) ICPL 151
(d) ICPL 317 (e) Prabhat (f) Pusa 18



Wilt of Arhar (*Cajanus cajan*)
(a) control, (b) *Fusarium oxysporum* (c) *Fusarium solani*

PLATE 10





Wilt of Arhar (*Cajanus cajan*)

(a) *Fusarium oxysporum* 1-control, 2-T7, 3-T17, 4-Bahar

(b) *Fusarium solani* 1-ICPL317, 2-GAUT82-58, 3-Bahar, 4-Pant A8505

Seedling rot of Arhar (*Cajanus cajan*)

(c) *Fusarium acuminatum* 1-control, 2-UPAS120, 3-Prabhat, 4-T21

(d) *Fusarium equiseti* 1-Pusa85, 2-AL15, 3-Pusa851, 4-Bahar

(e) *Fusarium* spp. 1-ICPL83006, 2-ICPL151, 3-C11, 4-Pant A8508